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THE EFFECTS OF PHOSPHORUS DEFICIENCY ON CITRUS[1,2]

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INTRODUCTION

INFORMATION CONCERNING the effects of mineral deficiencies and excesses on plants has proved of great value in the diagnosis of nutritional disorders in the field and has also provided many clues as to the function and interrelation of elements in plant metabolism and nutrition. In the case of citrus, knowledge of this subject, though extensive, is far from complete. Certain deficiencies, for example, have never been seen or produced on bearing trees; nor is it known, in many instances, which of the effects of a deficiency are primary and which secondary. Investigations concerned with various phases of citrus nutrition have led to the realization that a more thorough understanding of this subject is indispensable—is, in fact, a necessary cornerstone for further effective work. There are indications, too, that certain obscure physiological disorders affecting fruit production and fruit quality may be related to nutrition. Hence considerable experimental work has been carried out and is under way to extend our knowledge of the incipient and acute effects of deficiencies and excesses of mineral elements on the various species of citrus.

In connection with a soil-fertilizer experiment with young navel-orange trees in large containers (55-gallon oil drums), acute phosphorus deficiency developed in one of the soils used. Since, to the knowledge of the authors, the effects of a lack of this element on bearing orange trees have never been described, an account of the onset and progressive stages of this disorder is set forth herein.

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EXPERIMENTAL PROCEDURE

The fertilizer experiment referred to was begun in January, 1934, to determine whether large variations in the nitrogen, phosphorus, and potassium supply of soils would produce measurable effects on fruit quality.

Differential fertilizer treatments, as shown in table 1, were given duplicate cultures of each of two soils—one a calcareous Hanford fine sandy loam of low phosphate availability, from Santa Ana, California;

TABLE 1

DIFFERENTIAL FERTILIZER TREATMENTS GIVEN SOILS IN OIL DRUMS

Hanford fine sandy loam cultures	Sierra loam cultures	Fertilizer treatment	Amounts applied					
			Nitrogen as N		Phosphorus as P_2O_5		Potassium as K_2O	
			Per oil drum	Rate per acre*	Per oil drum	Rate per acre	Per oil drum	Rate per acre
			grams	*lbs.*	*grams*	*lbs.*	*grams*	*lbs.*
1 and 2	13 and 14	None	0.00	0	0.0	0	0.0	0
3 and 4	15 and 16	N (calcium nitrate)	13.75	482	0.0	0	0.0	0
5 and 6	17 and 18	NP (calcium nitrate and dicalcium phosphate)	13.75	482	136.5	4,784	0.0	0
7 and 8	19 and 20	NK (calcium nitrate and potassium sulfate)	13.75	482	0.0	0	33.8	1,185
9 and 10	21 and 22	NPK (calcium nitrate, dicalcium phosphate, and potassium sulfate)	13.75	482	136.5	4,784	33.8	1,185
11 and 12	23 and 24	NPK (calcium nitrate, dicalcium phosphate, and potassium sulfate)	13.75	482	273.0	9,568	33.8	1,185

* Rate per acre on area basis; the soil-surface area in the oil drums was 2.74 sq. ft.

the other a virgin Sierra loam containing ample available phosphate, from the University of California Citrus Experiment Station at Riverside. Enough of each soil was obtained to fill twelve 55-gallon containers, each soil being thoroughly mixed before filling the containers. The fertilizers used were calcium nitrate, dicalcium phosphate, and potassium sulfate. The phosphate and potassium sulfate were subsequently mixed throughout the soil of each of the cultures receiving these treatments. The nitrate was applied in solution to the top of the soil. At frequent intervals in the course of this experiment, subsequent applications of nitrate were given to those cultures receiving nitrogen, but no further phosphate or potassium fertilizer was added, save a surface application of dicalcium phosphate to the soil in culture 4 later in the experiment when this tree had become phosphorus-deficient; this was for the pur-

pose of testing the diagnosis. The cultures were watered with distilled water. The experiment was set up in a screened enclosure out of doors.

Oats were grown in the containers during the first year (1934) in order to provide preliminary information on responses to the fertilizer.

On March 4, 1935, one-year-old budded navel-orange trees, especially selected for uniformity, were planted in the containers. The appearance of the trees in the Hanford fine sandy loam, three months after transplanting, is shown in figure 1. Tomatoes were also grown in the containers at this time to determine whether the phosphate which had been

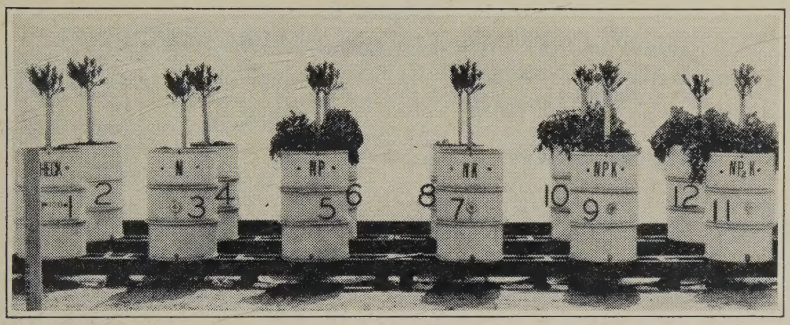

Fig. 1.—Young navel-orange trees three months after transplanting in differentially fertilized cultures. Two replicates. Fertilizer treatment was as follows: cultures 1 and 2, no treatment; cultures 3 and 4, calcium nitrate; cultures 5 and 6, calcium nitrate and dicalcium phosphate; cultures 7 and 8, calcium nitrate and potassium sulfate; cultures 9 and 10, calcium nitrate, dicalcium phosphate, and potassium sulfate; cultures 11 and 12, same as that for 9 and 10 save that twice as much dicalcium phosphate was used in these cultures. Note failure of interplanted tomatoes to grow in cultures which received no phosphate treatment.

applied seventeen months previously was still effective. Figure 1 shows that the added phosphate was still available and also demonstrates the extreme unavailability of the native phosphate of this soil for this plant; practically no growth was made in those cultures which received no phosphate. Subsequent trials with interplanted tomatoes gave similar results.

DEVELOPMENT AND DIAGNOSIS OF PHOSPHORUS DEFICIENCY

During the first three years (March, 1935, to March, 1938), no significant differences in growth of the citrus trees resulted from the differential fertilizer treatments in the Hanford soil, save for nitrogen deficiency in those cultures not receiving nitrate.[5] The green fruits which

[5] This was true in the case of the Sierra loam soil as well. Subsequently the trees in the Sierra loam soil developed an acute sulfur deficiency, the effects of which are described in the succeeding paper (*6*).

set in 1936 were picked; fruits which set in 1937, 1938, 1939, and 1940 were allowed to remain on the trees and ripen.

After the spring bloom in 1938, the four trees growing in the Hanford soil and receiving the nitrogen or nitrogen and potassium treatments (cultures 3, 4, 7, and 8) began to shed an abnormal number of leaves, as compared with the trees receiving phosphate. It was further noted that many of the falling leaves had burned areas and were of a dull-green color with a bronze cast. The low availability of the phosphate of

TABLE 2

PHOSPHATE IN HANFORD SOIL AFTER FOUR YEARS' CROPPING, COMPARED WITH PHOSPHATE OF ORIGINAL SOIL

Soil sample tested	Fertilizer treatment*	Phosphate ($PO_4^{\equiv}$) in dry soil	
		Water-soluble†	Acid-soluble‡
		p.p.m.	*p.p.m.*
Original soil	None	0.37	131.4
Culture:			
1	None	0.00	.....
2	None	0.00	.....
3	N	0.00	121.0
4	N	0.00	121.0
5	NP	24.50	.....
6	NP	24.50	.....
7	NK	0.00	.....
8	NK	0.00	.....
9	NPK	25.70	.....
10	NPK	24.50	.....
11	NPK	24.50	.....
12	NPK	22.30	.....

* For explanation of fertilizer treatment see table 1 (p. 162).

† Determination on 100 milliliters of a 1:5 water extract by the blue colorimetric method.

‡ Determination by the Truog (*17*) method; these tests were run only on the original soil and on cultures 3 and 4.

this soil immediately suggested phosphorus deficiency as the possible cause. Analyses of the woody tissue of one of these trees (no. 3) for inorganic phosphate (*5*)[6] showed only 23 p. p. m. $PO_4^{\equiv}$, on a green-weight basis, whereas similar tissue from a phosphate-treated tree (no. 5) contained 250 p. p. m. $PO_4^{\equiv}$. Determination of total phosphorus in burned and abscised leaves from tree no. 3 showed 0.07 per cent as against 0.13 per cent in comparable leaves taken from tree no. 15 growing in the Sierra loam soil, which received the same fertilizer treatment (calcium nitrate) but had none of the phosphorus-deficiency symptoms.

Soil samples taken from all the Hanford soil cultures on September 29, 1938, together with a sample of the original soil, were tested

[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

for water-soluble phosphate. Determinations of acid-soluble phosphate, made by the Truog (*17*) method, were also run on the original soil and on samples from cultures 3 and 4. The results of these tests are presented in table 2. Although the original soil contained a measurable amount of water-soluble phosphate, no trace was found in those cultures which did not receive phosphate treatment. On the other hand, there was almost as much acid-soluble phosphate present in cultures 3 and 4 as in the original uncropped soil, which indicates that the citrus trees had not materially reduced the reserve phosphate supply of this soil.

TABLE 3

COMPARATIVE CROSS-SECTIONAL AREAS OF TRUNKS OF NAVEL-ORANGE TREES GROWN IN DIFFERENTIALLY FERTILIZED SOILS, 1937–1940

Trees	Fertilizer treatment*	Average cross-sectional area of tree trunks†				
		Dec. 28, 1937	Sept. 24, 1938	Oct. 14, 1939	May 17, 1940	Net increase 1937–1940
		sq. cm	*sq. cm*	*sq. cm*	*sq. cm*	*sq. cm*
1 and 2	None	6.9	8.1	8.5	8.7	1.8
3 and 4	N	9.6	11.9	13.7	14.2	4.6
5 and 6	NP	9.6	13.4	16.2	16.1	6.5
7 and 8	NK	9.7	13.6	15.6	15.8	6.1
9 and 10	NPK	9.2	13.2	16.7	17.7	8.5
11 and 12	NPK	9.6	14.1	17.4	18.6	9.0

* For explanation of fertilizer treatment see table 1 (p. 162).

† Figures are averages of measurements at three permanently marked points (see fig. 2) on trunks of two trees in duplicate cultures.

Further evidence that the malnutrition noted was phosphorus deficiency was provided by the fact that phosphate applications to the surface of the soil of culture 4 in the summer of 1939 brought about definite tree recovery.

EFFECT OF PHOSPHORUS DEFICIENCY ON GROWTH AND APPEARANCE OF TREES AND ON FRUIT

The average cross-sectional areas of the trunks of the differentially fertilized trees in duplicate cultures, at various periods from December, 1937, to May, 1940, are shown in table 3. The measurements in 1937 were made before any symptoms of malnutrition had become evident. All save the nitrogen-deficient trees had made a very uniform growth up to this time. The subsequent retarded growth of the phosphorus-deficient trees is definitely shown by these data. This is more strikingly brought out by pictures of trees 3 and 5, taken in the spring of 1939 (plate 2, *A* and *B*). Color pictures of trees 3 and 5, taken a year later (plate 1), show the continued decline of the phosphorus-deficient tree.

Weak and limited new growth, premature abscission of older leaves, dieback of weakened twigs, together with a dull-green to bronze color of the foliage, were the more common features of this disorder. The leaves were small, somewhat thickened, and stood more upright in relation to the stem than normal leaves. No unusual twig, trunk, or root symptoms, such as splitting or gumming, have been observed to date.

Perhaps the best diagnostic symptom, though by no means the most conspicuous, is a burn which occurs on the older leaves. This is most pronounced in the spring after the emergence of the blossoms and new foliage.

From studies of phosphorus-deficient lemon plants grown from cuttings in the greenhouse in solution cultures, as well as from the aforementioned observations on bearing trees, it appears that the burn and premature abscission of older leaves takes place most prominently during periods when active terminal growth is being made. A seventeen-month-old, phosphorus-deficient lemon plant and a healthy lemon plant of the same age, both grown in the greenhouse, are shown in figure 2. Note that the older leaves of the phosphorus-deficient plant have been shed and, also, that leaf size is somewhat reduced. At the time these pictures were taken, new terminal growth was continuing to appear on the plant lacking phosphorus, and the older leaves along the lower stem were, concurrently, being shed. Many, though by no means all, of the falling leaves showed burn; all had a bronzed, lusterless appearance. The effects of phosphorus deficiency on lemon plants grown in the greenhouse agree with those described by Haas (*10*) for similarly grown citrus plants.

Apparently, there is a translocation of phosphorus from the older to the developing leaves as the supply of phosphate becomes deficient. Tests for inorganic phosphate at various points along the stem of the phosphorus-deficient lemon plants grown in the greenhouse showed much higher amounts at the growing point than toward the base. The total phosphorus content of old leaves shed at a point 36 inches up the stem from the base of the plant was 0.062 per cent; that of leaves 72 inches up the stem was 0.075 per cent; while that of green terminal leaves, 96 inches from the base, was 0.15 per cent. Corresponding leaves from healthy plants of similar age showed a total phosphorus content ranging from 0.15 to 0.25 per cent.

The burn noted on the leaves of the phosphorus-deficient trees often started as a discoloration (plate 1, *E*), giving them somewhat of a water-soaked appearance. Shortly thereafter, this area died completely (plate 1, *F* and *G*). At this stage, the injury resembled certain types of salt burn; in fact, so far as appearance is concerned, leaves burned at the

tip through phosphorus deficiency are indistinguishable from those injured by chloride. But, whereas the burned phosphorus-deficient leaves occur in greatest abundance in the spring, salt injury is more commonly seen in the fall and winter.

Fig. 2.—Seventeen-month-old lemon plants grown (*A*) in phosphate-deficient solution and (*B*) in complete nutrient solution. Note premature abscission of old leaves on the phosphorus-deficient plant. Both plants were grown in complete nutrient solution for thirteen months, after which the plant on the left (*A*) was deprived of phosphate.

Although the four phosphorus-deficient navel-orange trees (nos. 3, 4, 7, and 8) blossomed profusely in the spring of 1938, no fruit was produced, in contrast to a good set of fruit on the six trees receiving phosphate. Very weak, sparse bloom has characterized these trees in subsequent years, and the spring vegetative cycle has been limited (plate 1, *D* and *C*). The failure of these trees to bear fruit during the three years after they became phosphorus-deficient is clearly shown in table 4. A record of the fruit produced on all the differentially fertilized trees of this experiment, from 1935 to 1941, is set forth in this table.

Various quality studies were made on the fruits borne by the trees in all cultures in the year 1937–38, just preceding the onset of the phosphorus deficiency in cultures 3, 4, 7, and 8. The fruits were picked on

TABLE 4

NUMBER OF FRUITS BORNE BY NAVEL-ORANGE TREES GROWN IN DIFFERENTIALLY FERTILIZED HANFORD SOIL CULTURES, 1935–1941

Trees	Fertilizer treatment*	Total number of fruits on trees in replicate cultures					
		1935–36	1936–37†	1937–38	1938–39	1939–40	1940–41‡
1 and 2	None	0	0	0	0	0	0
3 and 4	N	0	9	10	0	0	0
5 and 6	NP	0	1	10	20	20	109
7 and 8	NK	0	6	13	0	2	0
9 and 10	NPK	0	9	10	37	37	46§
11 and 12¶	NPK	0	3	4	5	28	50

* For explanation of fertilizer treatment see table 1 (p. 162).

† Fruits picked green June 22, 1936.

‡ Green fruits on trees August 19, 1940.

§ Fruits on tree 10 only; tree 9 was harvested earlier.

¶ These two trees showed periodic symptoms of malnutrition, owing to the heavy phosphate applications given at the beginning of the experiment, and fruit production on these trees was subnormal.

February 4, 1938, and while at this time no symptoms of malnutrition were evident in the non-phosphate-treated trees, it was just after the spring blossom that the abnormally heavy leaf fall referred to took

TABLE 5

CHARACTERISTICS OF MATURE NAVEL ORANGES PRODUCED ON TREES GROWN IN DIFFERENTIALLY FERTILIZED CULTURES, 1937–38

Trees	Fertilizer treatment*	Total fruits produced and tested	Color of rind	Average rind thickness, percentage of total diameter	Average percentage of juice	Average total solids, degrees Brix at 17.5° C	Average anhydrous citric acid in juice	Average total phosphorus in juice
		number		*per cent*	*per cent*	*° Brix*	*per cent*	*per cent*
1 and 2	None	0	.....	...	....	....	....	.....
3 and 4	N	10	Deep orange	9.7	36.7	13.2	1.14	0.029
5 and 6	NP	10	Yellow orange	8.1	40.5	13.5	0.89	.064
7 and 8	NK	13	Deep orange	8.2	37.8	13.2	1.05	.036
9 and 10	NPK	10	Yellow orange	7.6	42.0	13.3	0.77	.072
11 and 12	NPK	4	Yellow orange	7.0	39.8	13.1	0.92	0.078

* For explanation of fertilizer treatment see table 1 (p. 162).

place. Since these trees must have been in the incipient stage of phosphorus deficiency at this time, the character of the fruit which matured is perhaps suggestive. The more pertinent data are reported in table 5 and show that fruits borne by the trees receiving no phosphate were characterized by a deeper orange color, thicker rind, less juice, higher

acid, and lower phosphorus content than the fruits from the phosphate-treated trees. The potassium treatment also apparently reduced rind thickness somewhat.

Only two fruits have since been produced on the phosphorus-deficient trees (table 4). Like the earlier ones, these fruits were deep orange in color. They had thick, coarse rinds, were decidedly lacking in juice, and were puffy. The fruits from the phosphate-treated trees during this same year, examined on the same date, had much thinner and smoother rinds, were very juicy, and showed no puffiness. More data will be needed to characterize definitely the effects of phosphorus deficiency on citrus fruit, but the preceding information is suggestive and fits in with existing evidence as to the influence of phosphorus on fruit quality (*1, 2*).

MINERAL COMPOSITION OF PHOSPHORUS-DEFICIENT ORANGE TREES

In order to characterize further the effects of phosphorus deficiency, inorganic analyses were made of various parts of phosphorus-deficient tree no. 8 and of healthy tree no. 9. These two trees were removed from the cultures in July, 1940. Samples of leaves, pencil-sized twigs, trunks, pencil-sized roots, and fine roots were washed in tap water and rinsed in distilled water. The bark was separated from the twigs, trunk parts, and pencil-sized roots; the interior woody parts were ground in a pencil sharpener while still green; and the bark, leaves, and fine roots, when air-dry, were ground in a Wiley mill. The samples were dried at 105° C, and analyses were made according to accepted procedures. The results are shown in table 6.

All parts of the phosphorus-deficient tree were low in phosphorus. The greatest contrast in the total phosphorus of the deficient and healthy plants was found in the bark and wood of the twigs, trunk, and coarse roots; the least difference was found in the young leaves. The older leaves were lower in phosphorus than the young leaves. These results indicate that the bark or woody tissue is more expressive as regards phosphorus status and more critical for diagnostic purposes than the leaves.

With the exception of the trunk wood, the nitrogen content of all parts of the phosphorus-deficient tree was higher than that of corresponding parts of the healthy plant. The differences in nitrogen content were most pronounced in the old leaves and the twig bark; and while the differences in the young leaves, interior root wood, and fine roots are small, they are probably significant. This is in harmony with the findings of many other investigators, who have shown that phosphorus-deficient plants are high in nitrogen and that nitrogen-deficient plants are high in phosphorus.

The potassium content of the young and old leaves and of the fine roots taken from the phosphorus-deficient tree was also higher than that of corresponding parts of the healthy tree; but in the other plant parts, the condition was just the reverse. The calcium and ash contents of the

TABLE 6

COMPARATIVE INORGANIC COMPOSITION OF PARTS OF PHOSPHORUS-DEFICIENT AND HEALTHY NAVEL-ORANGE TREES

Part of tree and condition	Constituents of dry matter, at 105° C								
	Ash	Ca	Mg	K	Na	Cl	N	P	S
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Young leaves:									
Phosphorus-deficient	12.55	2.84	0.18	2.56	0.08	0.19	3.46	0.14	0.26
Healthy	14.23	4.34	.12	1.55	.02	.39	3.38	.18	.23
Old leaves:									
Phosphorus-deficient	15.63	4.14	.18	2.50	.06	.21	5.00	.05	.23
Healthy	22.80	8.17	.09	0.80	.04	.35	1.70	.11	.26
Twig bark:									
Phosphorus-deficient	12.76	4.23	.08	0.43	.08	.09	3.03	Trace	.11
Healthy	15.47	5.22	.12	0.62	.05	.14	1.65	.28	.27
Twig wood:									
Phosphorus-deficient	4.89	1.73	.05	0.17	.05	.11	0.85	Trace	.09
Healthy	4.12	1.26	.08	0.24	.04	.14	0.72	.22	.12
Trunk bark:									
Phosphorus-deficient	12.41	3.20	.44	0.51	.06	.09	1.97	Trace	.63
Healthy	13.15	4.40	.35	0.66	.05	.11	1.64	.24	.18
Trunk wood:									
Phosphorus-deficient	3.26	1.13	.06	0.15	.05	.14	0.46	Trace	.22
Healthy	2.49	0.69	.08	0.21	.03	.14	0.60	.16	.11
Root bark:									
Phosphorus-deficient	9.22	2.79	.17	0.52	.08	.32	2.66	.01	.11
Healthy	11.00	3.26	.18	0.75	.02	.40	2.15	.24	.20
Root wood:									
Phosphorus-deficient	2.37	0.77	.07	0.06	.05	.11	0.70	.01	.05
Healthy	2.64	0.73	.09	0.18	.05	.12	0.66	.16	.08
Fine roots:									
Phosphorus-deficient	18.40	4.32	.22	0.75	.02	.35	2.03	.12	.12
Healthy	28.23	4.46	0.22	0.59	0.04	0.32	1.95	0.25	0.14

young and old leaves and of the twig, trunk, and root bark, as well as that of the fine roots, of the phosphorus-deficient tree were definitely lower than that of corresponding parts of the healthy tree; but that of twig and trunk wood was somewhat higher. There was no significant difference in the calcium or ash of the root wood. The differences in magnesium content of parts of the two trees were small; the greatest differ-

ences were found in leaves and in trunk bark, the phosphorus-deficient parts showing the higher content. In most cases, the sodium, chlorine, and sulfur contents were not much affected.

The burn which occurs on many of the older leaves and which, in the case of some leaves, is indistinguishable from chloride injury, is clearly not the result of chloride accumulation. The high nitrogen and potassium content of these old leaves, coupled with the observations of Eckerson (*8*), that phosphorus-starved plants store nitrate, suggests that the burn may be a result of excessive potassium nitrate accumulation. This possibility is being explored. Breakdown and disorganization of the cell protoplasm were noted by Reed (*16*) and Eckerson (*8*) in their studies of the effects of acute phosphorus deficiency.

DEVELOPMENT OF MANGANESE-DEFICIENCY SYMPTOMS ON PHOSPHORUS-DEFICIENT TREES

A further observation of considerable interest was the appearance of leaf symptoms of manganese deficiency on the summer-cycle growth of all the phosphorus-deficient trees in 1939 and again in 1940. When sprayed with manganese chloride, such leaves became green. A twig from a phosphorus-deficient tree, showing the typical manganese-deficiency leaf patterns (*7*) and the prompt recovery induced on a single leaf by painting with a dilute solution of manganese chloride, is presented in plate 2, *C*. The trees receiving phosphate showed no such symptoms.

Lyon (*13, 14*) has found that respiration and the production of carbon dioxide in green plants is pronouncedly increased by the use of phosphates, and Eckerson (*8*) has shown that reductase activity, as evidenced by nitrate accumulation, is decreased when phosphate is lacking. That phosphate is intimately linked with the vital activities of cells is indicated by the work of these and other investigators. It seems logical to infer that the appearance of manganese deficiency in the phosphorus-deficient trees of this experiment was owing to the decreased respiration of plant roots, which limited the production of carbon dioxide accordingly and consequently diminished solvent action on the sparingly soluble manganese compounds of this calcareous soil. That the manganese deficiency noted is a result of decreased solvent action of plant roots rather than a result of failure to utilize manganese after it has gained entrance into the plant is indicated (1) by the fact that manganese applications to the leaf brought about recovery; and (2) by the observations that the spring growth, in contrast to that of the summer and fall cycles, in 1939 and again in 1940, showed no manganese-deficiency symptoms. The trees were evidently able to absorb and store enough manga-

nese during the winter period, when vegetative growth is at a minimum, to suffice for the spring cycle.

DISCUSSION

While the phosphorus-deficient citrus trees of this experiment showed none of the anthocyanin pigmentation which is common on the stems and leaves of many plants lacking phosphorus (*3, 8, 9, 11, 12, 15, 18*), many characters similar to the effects of a deficiency of this element on other plants were apparent. Of these, greatly reduced growth rate, small leaves, lack of branching, continued terminal growth (weak and slow, however) at the expense of the older leaves, and bronze or dull-green color of old leaves were the most evident.

On tobacco, Karraker and Bortner (*12*) and McMurtrey (*15*) describe a necrotic spotting of the older leaves caused by phosphorus deficiency, though McMurtrey states that this character does not always develop. With citrus, the burn on older leaves occurs chiefly during periods when terminal growth is being made. Despite the somewhat irregular advent of this injury, it is perhaps the most diagnostic symptom; for sparse growth, open trees, and dull-green leaves may result in citrus from other causes also. Though the burn on some leaves resembles chloride injury, the latter occurs more commonly in the fall and winter, while the necrosis due to the lack of phosphorus is more prominent in the spring, after the emergence of the bloom and new-cycle growth.

Leaf analysis provides a fairly reliable means of distinguishing between the two injuries, since chloride-injured leaves show accumulations of chloride, whereas leaves burned as a result of phosphorus deficiency show no chloride accumulation and are distinctly subnormal in phosphorus content. Analyses of various parts of trees lacking phosphorus have also shown that the bark and woody tissue of pencil-sized twigs are exceedingly low in both total and inorganic phosphorus. These tests, together with the other symptoms described, would appear to be sufficient for diagnostic purposes when the deficiency is acute. As in other deficiencies, however, confirmatory tests, such as soil and plant treatments with phosphorus, should be undertaken as a final check.

The development of acute phosphorus deficiency in citrus grown in soil cultures has raised the question as to the possible phosphate needs of citrus grown under field conditions on comparable soils. A recent survey has been made of commercial citrus groves located on soils similar to the Hanford sandy loam used in this experiment. None of the trees in these groves showed any of the symptoms of phosphorus deficiency herein described, and tests for inorganic phosphate in the woody tissue of a number of the trees showed definitely higher amounts than were

found in the phosphorus-deficient trees of the experimental cultures. The acute deficiency which developed under the conditions of this experiment is probably accounted for by the restricted volume of soil available for root development. Under field conditions, with a much larger body of soil available for root growth, it is unlikely that acute phosphorus deficiency would develop. Moreover, the soils of the majority of commercial orchards in California (*4*) have been found to contain substantial accumulations of phosphate, owing to the past use of manures and mixed fertilizers. The continued use of manures or bulky organic materials will no doubt supply adequate phosphate for citrus needs, even though the phosphate of the original soil may be somewhat low.

SUMMARY

In connection with a fertilizer experiment on a calcareous Hanford fine sandy loam with young navel-orange trees in 55-gallon containers, an acute phosphorus deficiency developed in those trees receiving nitrogen or nitrogen and potassium but no phosphate.

The onset of this disorder was sudden. An abnormal shedding of leaves, which occurred just after the spring bloom in 1938, three years after planting, was the first indication of malnutrition. Some of the leaves showed burned areas, and many had a dull-green, bronzed, lusterless appearance. Little new growth was made subsequently, and the leaves were somewhat undersized, though not conspicuously so. Spring blossoms in the two succeeding years (1939 and 1940) were meager, and fruit failed to set, save for one fruit each on two trees during the year 1939. These two fruits were small in size, some puffiness was evident, and the juice content was low. With the exception of some dieback, no abnormal twig, trunk, or root symptoms developed. The inorganic and total phosphorus contents of all parts of the tree were subnormal; phosphorus in the bark and woody tissue was especially low. Fruit which matured on the phosphorus-deficient trees just prior to the development of leaf symptoms had a deeper orange color, thicker rind, and less juice than the fruit on the phosphate-treated trees.

A secondary manganese deficiency developed in the phosphorus-deficient trees. This was thought to be the result of the decreased solvent power of plant roots for the sparingly soluble manganese compounds of this soil, occasioned by diminished root respiration.

A survey of the trees of commercial citrus groves located on soils comparable to that used in this experiment showed no symptoms of phosphorus deficiency. Probably the deficiency which occurred in the experimental cultures resulted in part from the restricted root development owing to the limited quantity of soil available for root expansion.

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PLATES

Plate 1.—Phosphorus deficiency of navel-orange tree, shoots, and leaves: *A*, five-year-old phosphorus-deficient tree; *B*, healthy tree, same age; *C*, healthy shoot; *D*, phosphorus-deficient shoot showing small, bronzed, old leaves, lack of bloom, and weak new-cycle growth; *E–G*, phosphorus-deficient leaves, dull green to bronze in color, showing various types of burn on old leaves.

Plate 2.—*A*, Four-year-old phosphorus-deficient navel-orange tree (no. 3), showing reduced growth, sparse foliage, dead wood, and lack of fruit. *B*, Healthy tree of like age (no. 5). Both photographed February, 1939. *C*, Manganese-deficient navel-orange leaves which developed on the summer-cycle growth of a phosphorus-deficient tree. The tagged leaf became green less than a month after being painted with a solution of manganese chloride containing 5 milligrams of manganese per milliliter.

THE EFFECTS OF SULFUR DEFICIENCY ON CITRUS

H. D. CHAPMAN AND S. M. BROWN

THE EFFECTS OF SULFUR DEFICIENCY ON CITRUS[1,2]

H. D. CHAPMAN[3] AND S. M. BROWN[4]

INTRODUCTION

IN A PRECEDING PAPER (*3*)[5] an account is given of the development of phosphorus deficiency in citrus trees growing in one of two soils potted in 55-gallon containers. In the other soil an acute deficiency of sulfur occurred. The purpose of this paper is to describe the effects of this sulfur deficiency on the growth, appearance, fruit characters, and inorganic composition of the orange trees of this experiment. To the knowledge of the authors, sulfur deficiency of citrus trees growing in the field has never been recognized or described. Haas (*7*) has given a very brief description of sulfur deficiency of young Valencia trees grown in sand cultures. He states that this deficiency caused a chlorosis of the leaves. Total sulfur determinations in the leaves, twigs, root bark, and rootlets showed less of this constituent in the plant grown without sulfate than in corresponding plants of the same age growing in an adjacent nursery. The leaf symptoms illustrated, however, are unlike those produced on the experimental plants described in this paper.

EXPERIMENTAL PROCEDURE

The technique used in these experiments, as regards culture, differential fertilization, number of containers, and preliminary cropping, has been given in the accompanying paper (*3*), and only such details as appear necessary to an understanding of this paper are set forth herein.

The soil in which sulfur deficiency developed was obtained from a sagebrush-covered hillside on the property of the University of California Citrus Experiment Station at Riverside. It was derived from granite and is classified as a Sierra loam. This soil was initially used for purposes of comparison with the phosphorus-deficient Hanford fine sandy loam. Previous pot tests on the Sierra soil, while showing a low supply of total and available nitrogen, had given no hint of other deficiencies.

[1] Received for publication December 3, 1940.

[2] Paper no. 437, University of California Citrus Experiment Station, Riverside, California.

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[5] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

As noted in the paper on phosphorus deficiency (*3*, table 1), enough soil was obtained to fill twelve 55-gallon containers. Six treatments in duplicate were accorded this soil, as follows: cultures 13 and 14, no treatment; cultures 15 and 16, calcium nitrate; cultures 17 and 18, calcium nitrate and dicalcium phosphate; cultures 19 and 20, calcium nitrate and potassium sulfate; cultures 21, 22, 23, and 24, calcium nitrate, dicalcium phosphate, and potassium sulfate. The dicalcium phosphate and potassium sulfate were mixed throughout the soil at the rate of 4,784 pounds of P_2O_5, and 1,185 pounds of K_2O per acre, save for cultures 23 and 24, which received P_2O_5 at the rate of 9,568 pounds per acre.[6] The calcium nitrate was applied in solution at the rate of 482 pounds per acre to the surface of the soil in the beginning, and frequent additions were made subsequently during the course of the experiment. No further applications of phosphate or potassium were made, but as noted later, subsequent additions of sulfur and of calcium sulfate were accorded to some of the cultures for diagnostic purposes. The cultures were watered with distilled water throughout.

After a preliminary cropping with oats, one-year-old budded navel-orange trees were planted in the containers on March 4, 1935.

DEVELOPMENT AND DIAGNOSIS OF SULFUR DEFICIENCY

While none of the orange trees in the Sierra loam grew quite so well as those in the Hanford fine sandy loam, little effect from fertilizer additions was evident for the first three years save for extreme nitrogen deficiency in cultures 13 and 14 and a very slight growth response from the potassium sulfate treatments in cultures 19, 20, 21, 22, 23, and 24. In the spring of 1938, however, the new growth on all the trees was distinctly yellowish. It was thought that this might be the result of insufficient aeration and failure of the plant roots to absorb adequate nitrogen; poor water penetration into this soil had been noticed almost from the beginning, free water often standing on the surface for several days after an irrigation.

To determine whether the physical state of this soil could be improved, 27 grams of sulfur per culture (equivalent to a rate of 946 pounds per acre on an area basis) was mixed into the top 2 or 3 inches of soil of half the replicated cultures of this series (nos. 16, 18, 20, 22, 24) on July 18, 1938. Within a few weeks, the yellow foliage of the sulfur-treated trees began to turn green; and shortly thereafter, healthy, vigorous new growth appeared. The untreated trees showed no improvement.

[6] Rate per acre calculated on an area basis. The soil-surface area in oil drums was 2.74 square feet.

Since the effects of sulfur on soils are diverse, and the results noted could have been caused by the improved physical condition of the soil or by the effects on nutrient availability, further experiments were undertaken.

To determine the characteristic effects of sulfur deficiency, a sand-culture experiment of the automatically operated type (*4*), using sweet-orange and grapefruit seedlings and lemon plants grown from cuttings, was begun in the greenhouse. One sand-culture unit was provided with a complete nutrient solution of a type known to be suitable for good citrus growth, and another with a sulfate-deficient nutrient solution in which the sulfate-carrying salts were replaced by nitrates. After a growing period of about six months, the terminal foliage of all plants in the cultures lacking sulfate became yellow, the affected leaves being more or less uniformly yellow, as in nitrogen deficiency. The older green leaves, however, retained their green color to a somewhat greater degree than when nitrogen is lacking. The appearance of these sulfur-deficient plants at this stage was strikingly similar to that of the navel-orange trees in the soil cultures, especially at periods following the emergence of new-cycle growth.

Soil samples taken from the soil cultures in September, 1938, were extracted with water, and tests for sulfate were made on the filtered solution. Substantial quantities were found in those soils which had been treated with sulfur, but only a trace in the untreated soils.

In the spring of 1939, the new-cycle growth on the non-sulfur-treated trees was again very yellowish, as in the previous year.

On June 21, 1939, several clusters of such yellowed leaves from tree 19 were sprayed with a 2-*N* solution of sodium sulfate. Within a few weeks some green spots appeared on these leaves, whereas there was no change in the untreated yellowed leaves.

On July 9, 1939, 28 grams of calcium sulfate (equivalent to a rate of 981 pounds per acre applied on an area basis) was applied to the surface of the soil of one of the chlorotic tree cultures (no. 19). In the course of the summer, the yellowish leaves of the tree in this culture became green, while the leaves of the untreated trees remained essentially unchanged.

In order to further verify the belief that the malnutrition of these trees was sulfur deficiency, total sulfur and nitrogen determinations were made on terminal yellow leaves and old green leaves picked from the affected navel-orange trees (no. 15), as well as on corresponding leaves from the sulfur-deficient sweet-orange seedlings grown in the greenhouse. For comparison, leaves of comparable age from one of the now healthy, sulfur-treated trees (no. 16), growing in Sierra loam,

from one of the nitrogen-deficient trees (no. 14) growing in this soil, and from control cultures growing in the greenhouse were also analyzed. The results are presented in table 1. The sulfur and nitrogen contents of the leaves of the navel-orange trees suspected of sulfur deficiency were very similar to those of the leaves of known sulfur-deficient plants grown under controlled conditions in the greenhouse. Despite the nearly identi-

TABLE 1

SULFUR AND NITROGEN CONTENTS OF LEAVES FROM HEALTHY AND YELLOWED CITRUS PLANTS

Source, age, and character of leaves tested	Composition of leaves, in percentage of dry matter	
	Total sulfur	Total nitrogen
	per cent	*per cent*
Sulfur-deficient sweet-orange seedlings grown in greenhouse:		
Young terminal yellow leaves	0.075	3.88
Old green leaves	.120	3.31
Healthy sweet-orange seedlings grown in greenhouse:		
Young terminal green leaves	.260	3.46
Old green leaves	.220	3.15
Chlorotic navel-orange trees grown in Sierra loam soil:		
Young terminal yellow leaves	.096	2.54
Old green leaves	.129	2.44
Nitrogen-deficient navel-orange tree grown in Sierra loam soil:		
Young terminal yellow leaves	.189	1.12
Old yellow leaves	.320	1.13
Healthy sulfur-treated navel-orange tree grown in Sierra loam soil:		
Young terminal green leaves	.202	2.15
Old green leaves	0.320	1.66

cal appearance of leaves affected by lack of nitrogen and those affected by lack of sulfur, it will be noted that the nitrogen content of the sulfur-deficient leaves is a little higher than that of healthy green leaves, whereas the sulfur content is, roughly, one half that of leaves from healthy plants.

In the spring and summer of 1940, the non-sulfur-treated trees (that is, those which had received neither sulfur nor calcium sulfate) again produced an extremely yellowish cycle of growth which was even more marked than in the two preceding years. On May 29, 1940, 100 grams of calcium sulfate was incorporated into the soil surface of another of the sulfur-deficient tree cultures (no. 17). In one month's time, the yellow leaves of this tree had become green. Subsequently, healthy new-cycle growth emerged, and this tree now stands in sharp contrast to the untreated trees.

All these observations prove that the malnutrition which developed in these trees was acute sulfur deficiency.[7]

EFFECT OF SULFUR DEFICIENCY ON TREE GROWTH AND FOLIAGE

As already mentioned, the onset of sulfur deficiency was shown by the appearance of a decidedly yellowish type of new growth. The typical appearance of young terminal leaves and of older leaves on the same

Fig. 1.—Shoot from a sulfur-deficient navel-orange tree (no. 15), showing yellow new-cycle leaves which stand in sharp contrast to the green older leaves. This type of growth is especially prominent in the earlier stages of sulfur deficiency. The yellow sulfur-deficient leaves are similar in appearance to nitrogen-deficient leaves. In many instances the midrib is somewhat more yellow than the rest of the leaf.

shoot, at the time when this disorder first became manifest, is shown in figure 1. The terminal growth was distinctly yellow, though there was no leaf pattern other than a tendency for the midrib to be a little more yellow than the mesophyll tissues. The chlorotic terminal growth stood in sharp contrast to the older green leaves during the first month or so

[7] It is curious that sulfur deficiency should have developed at the same time and to the same degree in cultures 19, 20, 21, 22, 23, and 24, which received potassium sulfate initially. One explanation is that most of the sulfate had been leached out of the surface by the frequent additions of distilled water before the trees were planted in this soil. As noted previously, a preliminary crop of oats was grown in these soils prior to planting the trees. Another possibility is that some of the sulfate may have been reduced and disappeared as hydrogen sulfide in the periods following an irrigation, when water often stood in these soils for several days at a time.

after the appearance of the new-cycle growth. As the yellow leaves aged, they gradually became somewhat greener, and the contrast with subsequent new cycles of growth was less conspicuous. The leaves became leathery and thickened and finally attained a dull-green color; the midribs on many were more yellow than the rest of the leaf.

The new growth which appeared in 1939 and 1940 was more yellow than that of the preceding year, and the leaves were smaller. The appearance of the sulfur-deficient tree no. 23, in June, 1939, is shown in plate 1, *A*. The dull-green color of the old leaves, many of them with a somewhat more yellowish midrib, is shown in plate 1, *C*, in contrast with leaves from a healthy tree (plate 1, *B*). The spring-cycle growth in 1940 consisted essentially of an exceedingly profuse though weak bloom, scarcely any leaves accompanying this bloom (plate 1, *D*). No fruit was set, and considerable dieback of these twigs subsequently took place. The cream-colored June-cycle growth which followed is shown in plate 1, *E*. The leaves were small and immature. Subsequently, with hot weather, considerable burn took place, both at the leaf tips and in other parts of the leaf. Such burn is not uncommon with citrus leaves which, for one cause or another, are lacking in chlorophyll. Many of these June-cycle leaves had dropped by September.

Save for considerable dieback, no abnormal twig, branch, trunk, or root symptoms, such as splitting or gumming, have occurred.

EFFECT OF SULFUR DEFICIENCY ON FRUIT

While only one of the sulfur-deficient trees (no. 21) bore fruit in 1940–41, most of them produced a few fruits each during the year 1939–40. All of these fruits had definite color characteristics in common. In place of the deep-green color of healthy immature fruits, those on the sulfur-deficient trees were of a light yellowish-green color throughout their early development, in this respect paralleling the chlorotic appearance of leaves (see plate 2, *A*). Maturing fruits started to turn color at about the same time as those on the healthy trees but failed to develop the orange color of the healthy fruit. Instead, they were of a distinctly lemon-yellow hue. Some of the fruits were small and misshapen; many of them attained normal size, however.

Examination of the interiors of affected fruits revealed, in many, an incomplete development of the juice vesicles and, in some, a distinct gelatinization of the contents. Most of such fruit had a somewhat thickened rind. Cross sections of healthy and sulfur-deficient fruits are shown in figure 2. Not all the fruits were so seriously affected as the one illustrated, but nearly all showed more or less rind thickening and some gelatinization of the juice-vesicle contents. The exterior appearance of

immature fruits and the exterior and interior of mature healthy and sulfur-deficient fruits are shown in plate 2. The similarity of some of these characters to the condition known as "granulation" (*2*) is rather marked. Whether there is any necessary connection is unknown.

Determinations of the acid and soluble-solids content of the juice of

Fig. 2.—Cross sections through center of mature (*A*) sulfur-deficient and (*B*) healthy navel oranges. Note thickened rind and shriveled juice vesicles of sulfur-deficient fruit. This is a somewhat extreme case; not all the fruit from the sulfur-deficient trees was so adversely affected (see plate 2).

mildly affected fruit revealed a low sugar content but no significant difference in acid, in comparison with healthy fruit of like age. There was a noticeable lack of oil in the rind of the sulfur-deficient fruit.

INORGANIC COMPOSITION OF SULFUR-DEFICIENT ORANGE TREES

In July, 1940, one of the sulfur-deficient trees (no. 15) was removed from the culture, and inorganic analyses were made. The methods of sampling and analyzing were identical with those described in the preceding paper (*3*). The results, compared with those obtained from analyses of similar parts of a healthy tree, are presented in table 2.

All parts of the sulfur-deficient tree were lower in sulfur than corresponding parts of the healthy tree. The greatest contrast in total sulfur in the two trees was found in the bark and wood of the twigs, trunk, and coarse roots. The younger leaves showed a lower sulfur content than the older leaves.

Total nitrogen of all parts of the tree lacking sulfur was higher than that of the healthy tree. This difference was especially marked in the old

leaves. Since there is a decided similarity in the appearance of sulfur- and nitrogen-deficient leaves, analysis affords a decisive means of distinguishing between them: the leaves of nitrogen-deficient trees, as

TABLE 2

COMPARATIVE INORGANIC COMPOSITION OF PARTS OF SULFUR-DEFICIENT AND HEALTHY NAVEL-ORANGE TREES

Part of tree and condition	Constituents of dry matter, at 105° C								
	Ash	Ca	Mg	K	Na	Cl	N	P	S
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Young leaves:									
Sulfur-deficient	16.02	3.56	0.22	3.40	0.210	0.35	4.90	0.50	0.050
Healthy	14.23	4.34	.12	1.55	.020	.39	3.38	.18	.230
Old leaves:									
Sulfur-deficient	15.40	5.05	.18	0.81	.100	.42	5.30	.34	.130
Healthy	22.80	8.17	.09	0.80	.040	.35	1.70	.11	.260
Twig bark:									
Sulfur-deficient	12.25	4.32	.13	0.34	.010	.12	3.03	.10	.010
Healthy	15.47	5.22	.12	0.62	.005	.14	1.65	.28	.270
Twig wood:									
Sulfur-deficient	5.31	1.89	.06	0.15	.010	.09	0.85	.01	.004
Healthy	4.12	1.26	.08	0.24	.004	.14	0.72	.22	.120
Trunk bark:									
Sulfur-deficient	12.83	4.23	.25	0.29	.005	.07	2.12	Trace	.010
Healthy	13.15	4.40	.35	0.66	.005	.11	1.64	.24	.180
Trunk wood:									
Sulfur-deficient	3.32	1.10	.07	0.17	.004	.12	0.73	Trace	.010
Healthy	2.49	0.69	.08	0.21	.003	.14	0.60	.16	.110
Root bark:									
Sulfur-deficient	8.75	2.56	.12	0.61	.006	.35	2.66	.22	.040
Healthy	11.00	3.26	.18	0.75	.020	.40	2.15	.24	.200
Root wood:									
Sulfur-deficient	1.78	0.52	.09	0.09	.009	.14	0.70	.06	.008
Healthy	2.64	0.73	.09	0.18	.005	.12	0.66	.16	.080
Fine roots:									
Sulfur-deficient	13.73	4.04	.21	0.54	.010	.39	2.81	.30	.080
Healthy	28.23	4.46	0.22	0.59	0.040	0.32	1.95	0.25	0.140

shown in table 1, are distinctly subnormal in nitrogen content and somewhat higher in total sulfur, whereas the reverse is true when the yellowing results from lack of sulfur.

With other mineral elements, results were not always consistent in different parts of the tree. The leaves and fine roots of the sulfur-deficient plant were distinctly higher in phosphorus content, than those of the healthy plant. The condition was reversed, however, in the bark

and wood of twigs, trunk, and coarse roots, the phosphorus content being distinctly lower in the sulfur-deficient than in the healthy plant parts, that of the trunk being as low as in phosphorus-deficient trees. The potassium content of the young sulfur-deficient leaves was abnormally high; but in the bark and woody tissue it was lower than in corresponding parts of the healthy tree. The calcium and total-ash content were, for the most part, lower in the sulfur-deficient than in the healthy tree.

In general, there is a decided parallelism in the nitrogen, potassium, calcium, and total-ash contents of these sulfur-deficient trees and the phosphorus-deficient trees discussed in a previous paper (*3*). One point of difference is the relatively lower sulfur content of young leaves as compared with old leaves of the sulfur-deficient tree. Under conditions of phosphorus deficiency, the young leaves are higher in phosphorus than the old leaves. This is in harmony with the observation that in sulfur deficiency the young growth is the first to be affected, whereas in phosphorus deficiency the older leaves are the first to show the effect.

DISCUSSION

The external effects of sulfur deficiency on bearing citrus trees agree in many respects with those described by other investigators on a wide range of plants. General yellowing of the foliage, especially of the terminal growth, and a resemblance to nitrogen deficiency are the more prominent characters emphasized. The similarity of symptoms of sulfur deficiency of citrus to those of tea plants, as reported by Storey and Leach (*10*), is marked: the undersized, yellow, uprolled, tipburned young leaves and their premature abscission followed by twig dieback, as seen on tea plants, are also characteristic of citrus trees. These investigators found that absorption of potassium sulfate, magnesium sulfate or sodium sulfate by cut shoots brought about prompt recovery. While this treatment has not as yet been tried with citrus trees, leaves sprayed with a solution of sodium sulfate developed green spots. Recovery after soil application of sulfate was rapid.

McMurtrey (*8*) noted on sulfur-deficient tobacco plants a yellowing of the leaf midrib and veins analagous to that seen on the citrus trees here described. In connection with vein yellowing, however, it should be noted that this frequently occurs in citrus leaves from other causes. Substantial root or bark destruction due to disease, gopher, or mechanical injury are common causes. The sulfur-deficient leaves which show this symptom, while of shorter life than healthy leaves, do not fall so early or abruptly as do leaves which become affected with the vein chlorosis caused by root rot or other troubles.

Though no studies of organic composition were made on the affected citrus trees, Nightingale, Schermerhorn, and Robbins (*9*) and Eaton (*5*), in studies of the metabolism of sulfur-deficient plants, found accumulations of carbohydrates, nitrates, and proteolytic products. Eckerson (*6*) noted that lack of sulfur decreases the reductase of soybean and tomato plants.

A thickening of cell walls of sulfur-deficient plants was found by Nightingale, Schermerhorn, and Robbins (*9*) and by Eaton (*5*). The thickened and leathery leaves which developed on the citrus plants may be a reflection of excessive lignin formation.

The parallelism between sulfur and phosphorus deficiencies, noted by the aforementioned workers as manifest in carbohydrate and nitrate accumulations, is also apparent in the inorganic composition of citrus plants affected by the two deficiencies. The promptness of the recovery of sulfur-deficient plants when sulfur is supplied is noteworthy and is no doubt owing, in part, to the accumulations of carbohydrate and nitrate, which are important foundation materials for the synthesis of proteins and other vital plant constituents.

The development of sulfur deficiency in citrus grown in Sierra loam cultures has raised the question whether commercial citrus orchards in any part of California might be lacking in this element. Considerable areas in certain parts of Oregon, Washington, and California are low in sulfur, and crops respond to additions of this element. Few citrus groves are likely to benefit by sulfate fertilization, however, for the following reasons. In the first place, all irrigation waters carry more or less dissolved sulfate; and while those waters derived from the runoff of the essentially granitic-type mountainous areas are low in sulfate content, the renewal is frequent, and citrus-tree requirements for sulfur are rather low.[8] Also, a certain amount of sulfur is brought down annually by rainfall. And Alway, Marsh, and Methley (*1*) have shown that air, even in regions remote from industrial centers, contains a small amount of sulfur dioxide, part of which is absorbed by the soil and by growing crops. In addition, any organic matter added to the soil in the form of manures, straws, and so forth, will furnish available sulfur, as will ammonium sulfate or mixed fertilizers carrying potassium sulfate or superphosphate. Pest-control operations employing dusting sulfur or sulfur-containing insecticides add to the sulfur supply of soil. Hence, even on citrus soils low in sulfur, deficiencies are not likely to develop under California conditions, except perhaps in isolated instances where waters of low-sulfate content prevail and no sulfur or sulfur-containing

[8] Computations based on analyses of whole fruits show that a yield of 20,000 pounds of fruit per acre would remove about 25 pounds of sulfur.

compounds are used in the commercial production of citrus. In conclusion, it should be noted that many California citrus soils and irrigation waters, for example, those of Imperial, Orange, and Ventura counties, are high in sulfate content.

SUMMARY

A condition of malnutrition which developed gradually in young navel-orange trees growing in a granitic-derived soil in large 55-gallon containers was found to be sulfur deficiency. This disorder was characterized by an abnormal yellowing of the new-cycle growth, similar to the more or less uniform yellowing caused by nitrogen deficiency. In many of the leaves, the midrib was somewhat more yellowish than the rest of the leaf.

In contrast to nitrogen-starved leaves, sulfur-deficient leaves had a higher nitrogen content than is normal for healthy green leaves and a lower sulfur content, whereas nitrogen-deficient leaves had a subnormal nitrogen content and a slightly higher sulfur content. Thus it is possible by leaf analysis to differentiate definitely between sulfur and nitrogen deficiency.

With the exception of considerable dieback, no abnormal twig or bark symptoms developed on the trees lacking sulfur. While growth was limited, as with phosphorus-deficient trees, a profuse, though weak, bloom was a characteristic feature. This may be a result of carbohydrate accumulation, since different workers have shown that one of the effects of sulfur deficiency in a number of plants is an accumulation of starch and other forms of carbohydrate.

In place of the deep-green color of healthy immature fruits, those produced on the sulfur-deficient trees were of a light yellowish-green color; and maturing fruits failed to develop the orange color characteristic of fruit produced on healthy trees. They were, instead, distinctly lemon yellow in color. Most of the sulfur-deficient fruit showed abnormally thick rinds and reduced juice content. In many of the fruits, the juice vesicles were shriveled; in the less severely affected fruit, the contents of many of the juice vesicles were gelatinized, as in granulation.

Inorganic analyses of leaves, twigs, trunk, and roots of a sulfur-deficient tree were made. The sulfur-deficient leaves showed, in general, a higher nitrogen, phosphorus, potassium, and magnesium content and a lower calcium and sulfur content than the leaves from a healthy tree of like age. Except for the young leaves, the ash content of all parts of the tree was less in the sulfur-deficient tree. A certain degree of parallelism in the composition of sulfur-deficient and phosphorus-deficient orange trees is apparent.

Though many western soils are low in total sulfur, it does not appear probable that, except in isolated instances, commercial citrus orchards would benefit by sulfate fertilization. Not only do irrigation waters carry more or less dissolved sulfate, but small increments are also brought down by rains; these supplies added to the sulfur or sulfur-bearing compounds used incident to fertilization and pest control probably more than meet citrus-tree requirements.

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PLATES

Plate 1.—Sulfur deficiency of navel-orange tree: *A*, four-year-old tree showing chlorotic new-cycle growth and dull-green old leaves (June, 1939); *B*, healthy shoot; *C*, sulfur-deficient shoot showing dull-green leaves with yellowish midrib and weak new-cycle spring growth; *D*, spring-cycle growth (1940) showing profuse but weak bloom; *E*, extremely chlorotic sulfur-deficient June-cycle growth which emerged after the spring bloom. (Note upright position on stem, small leaves, and tipburn.) These yellow leaves showed progressive burning on the tips and margins and, in some leaves, brown necrotic spots in mesophyll areas. Many of these June-cycle leaves had fallen by September.

Plate 2.—Sulfur-deficient and healthy navel oranges. Immature (six-month-old) fruit (*A*) from healthy tree and (*B*, *C*) from sulfur-deficient tree. The lemon-yellow color of mature sulfur-deficient fruit (*E*) is shown in contrast with the orange color of mature healthy fruit (*D*). Cross sections show gelatinization of contents of juice vesicles of sulfur-deficient fruit (*F*) in comparison with healthy fruit (*G*).

SPECIES OF STIGMINA AND STIGMELLA OCCURRING ON PLATANUS

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SPECIES OF STIGMINA AND STIGMELLA OCCURRING ON PLATANUS[1,2,3]

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INTRODUCTION

THE FOUR OR FIVE species of *Platanus*, or plane trees, commonly known as "sycamores" in the United States, occur widely throughout North America, Europe, and Asia. These trees are notable for their picturesque beauty in their natural habitats and are widely planted for use as ornamentals on lawns and along streets. Their foliage, however, is subject to attack by various fungi, among which are species of *Stigmina* and *Stigmella*. These closely related conidial fungi possess oval to oblong brown spores, whose distinguishing characteristics are the transverse septa in those of *Stigmina* sp. and the muriform septa in those of *Stigmella* sp. These fungi have been reported to cause the production of lesions on the leaves of *P. orientalis* L., *P. occidentalis* L., and *P. racemosa* Nutt. The diseases which they cause are not of major importance, for they do not seriously threaten the destruction of the trees. In certain localities, however, and in certain seasons, the leaf spots are conspicuously abundant, and affected trees are prematurely defoliated.

Most plant pathologists and mycologists who have collected specimens of *Stigmina* and *Stigmella* on *Platanus orientalis, P. occidentalis,* and *P. racemosa* have regarded the pathogens as one and the same species, that is, *Stigmina Platani* (Fckl.) Sacc. But, when this study was begun in December, 1935, at the University of California Citrus Experiment Station, it soon became apparent that the identity and nomenclature of these fungi were in a confused state. For this reason, a comparative study of *Stigmina* and *Stigmella* on *Platanus* was undertaken.

ORGANISMS INVOLVED

Three related but distinct fungi have been shown to be involved in these diseases: *Stigmella Platani-racemosae* Dearn. and Barth. *apud* Dearn. on *Platanus racemosa,* in California; *Stigmina Platani* (Fckl.)

[1] Received for publication October 28, 1940.

[2] Paper no. 438, University of California Citrus Experiment Station, Riverside, California.

[3] The major part of this paper is based on a thesis by the senior author submitted in partial fulfillment of the requirements for the degree of Master of Science, University of California, 1937. (Typewritten.) Copy on file in the Library of the University of California, Berkeley. The present paper includes further research undertaken, after the thesis was completed, in coöperation with the junior author.

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Sacc. on *P. orientalis,* in Europe; and a species of *Mycosphaerella* on *P. occidentalis,* in the southeastern and southern central United States.

The third fungus was described as a new species by Wolf (*24*)[6] and named *Mycosphaerella Stigmina-Platani,* in the belief that it was the perithecial stage of *Stigmina Platani.* This *Mycosphaerella* has an unnamed polymorphic conidial stage, some of whose conidia are typical of the genus *Stigmina,* others of *Cercospora,* and still others intermediate in shape between these two types. Polymorphism in the conidial stage is indicated by Wolf (*24,* fig. 8). The conidial stage of *Stigmina Platani* does not show polymorphism. The evidence from pathogenicity, appearance of the disease, physiological characteristics, and morphology of the conidial stage, discussed later in the paper, indicates that the conidia of the *Mycosphaerella* on *Platanus occidentalis* are distinct from those of *Stigmina Platani* and that the *Mycosphaerella* described by Wolf (*24*) is apparently not the perithecial stage of *Stigmina Platani.*

It seems advisable, therefore, to reject the name *Mycosphaerella Stigmina-Platani* Wolf as untenable, according to the *International Rules of Botanical Nomenclature* (*9*), which states:

> Art. 64. A name of a taxonomic group must be rejected if the characters of that group were derived from two or more entirely discordant elements, especially if those elements were erroneously supposed to form part of the same individual.

In its place, the name *Mycosphaerella polymorpha* is proposed, with the following description:

> *Mycosphaerella polymorpha* n. n.
> *Mycosphaerella Stigmina-Platani* Wolf (*24,* p. 60–61) *nomum confusum.*

Perithecia in vernale in putrescentibus foliis efformantia, hypophylla per totum folium dense dispersa, punctiformia, nigra, erumpenti-immersa, sphaeroidea, 65–85 μ diam.; ascis sacciformibus, fasciculatis, octosporis, aparaphysatis, 55–70 × 9–11 μ; sporidiis biseriatis, loculis inaequalibus, loculo superiore crassiore, hyalinis, rectis vel curvulis, 8–19 × 4–7 μ.

Spermogoniis autumno efformantibus, numerosis, hypophyllis, innatoprominulis, paginis inferioribus ex toto vel in maculis exaridis occupantibus, ovatis vel globosis, nigris, 55–65 μ; spermatiis bacilliformibus, 2–3 × 1 μ, hyalinis.

Hab. in foliis dejectis *Platani occidentalis.*

Status conidicus: Statum conidicum *Stigmina polymorpha* n. n. sistit. Caespitulis hypophyllis, atris, primo maculicolis deinde subeffusis; conidiis polymorphicis, stigminoideis vel cercosporoideis, 14–70 × 3–11 μ, intense olivaceis, 1–8-septatis, non-constrictis; conidiophoris fasciculatis, fuscidulis. Hab. stato conidico non modo in pagina inferiore *Platani occidentalis* parasitico sed in foliis vivis *Platani racemosae, P. Wrightii,* atque *P. acerifoliae,* in Amer. bor.

There remains, as will be indicated in this report, the possibility that the pathogen *Mycosphaerella polymorpha* on *Platanus occidentalis* has

[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

been confused with *M. platanifolia* (Cke.) Wolf. *M. platanifolia* was first described from leaves of *P. occidentalis* by Cooke (*3*) as *Sphaerella platanifolia,* and its conidial stage is stated by Wolf (*24* and *25*) to be *Cercospora platanicola* Ellis and Everhart (*5*).

Whether the organism identified by Saccardo (*20*) as *Stigmina Visianica* Sacc. is correctly named, has been questioned by Bubák (*2,* p. 219), who says:

> Bei einem amerikanischen Exemplare dieses Pilzes (Claremont bei Los Angelos in Süd-Kalifornien, *Platanus racemosa,* leg. Baker) finde ich sehr oft auch Sporen mit einer Längswand, so dass die Unterschiede zwischen *Stigmella* und *Stigmina* nicht allzu fest sind.
>
> Die zweite europäische Art *Stigmina Visianica* Sacc. ist von *St. Platani* nicht verschieden. Schon Lindau . . . . weist auf diesen Umstand hin. Ich finde die Sporen bei beiden genannten Arten sehr variabel und gleich gross. Demnach ist *Stigmina Visianica* Sacc. nur ein Synonym zu *Stigmina Platani* (Fuckel) Saccardo.

A specimen from the Farlow Herbarium, bearing the notation "*Stigmella Visianica* Sacc.? if it is different from *Stigmina Platani* f. Ellis. Pine Hills, Illinois, September 1884," was examined by the writers and found to be similar to the fungus on *Platanus occidentalis,* herein identified as *Mycosphaerella polymorpha.*

HISTORY AND DISTRIBUTION OF THE FUNGI

The name *Stigmina Platani* was employed by Saccardo in 1878 (*18*) and in 1880 (*19*) for an organism called, as early as 1815, *Puccinia Platani* Bivona. Later, Saccardo (*20*) used the name *Stigmina Platani* also to replace that of *Stigmella Platani* Fuckel, employed by Thümen (*22*) for the same organism. Specimens of this fungus had been sent from Greece to Thümen and transmitted by him to Fuckel, who, in 1873, named the fungus *Stigmella Platani.* That it occurs elsewhere in Europe is evidenced by the fact that Saccardo (*20*) records it from Germany, Bubák (*2*) from Tirol (Austria) and Istria (Italy), and Nattrass (*13*) from Cyprus.

The fungus now known as *Stigmella Platani-racemosae* was stated by Harkness (*7*), who collected it on *Platanus racemosa* near Niles, in Alameda County, California, to have been present in California as long ago as 1885; and its presence in this state was mentioned by McClatchie (*12,* p. 376) in 1897. Both Harkness and McClatchie called it *Stigmina Platani,* however, in the belief that it was identical with the organism occurring in Europe on *P. orientalis.* Apostolides (*1*), in 1929, studied this disease in California and also identified the causal fungus as *Stigmina Platani.* In the same year, Dearness and Bartholomew (Dearness, *4*) described this pathogen as *Stigmella Platani-racemosae,* recognizing

that the leaf-spot fungus on *P. racemosa* was distinct from *Stigmina Platani* and basing their description upon specimens collected at Riverside, California. Specimens in the Claremont College herbarium (Baker's collection no. 3956), identified as *Stigmina Platani* on *P. occidentalis,* were examined by the writers and found, also, to be *Stigmella Platani-racemosae* on *P. racemosa.*

To date, *Stigmella Platani-racemosae* is not known to occur outside of California. Through correspondence with interested collectors, the writers have determined that the fungus is present in the following counties in southern California: San Diego, Los Angeles, Orange, Riverside, San Bernardino, Santa Barbara, and Ventura. It has not been collected in the northern part of the state except by Harkness (*7*).

The fungus *Mycosphaerella polymorpha* is of widespread occurrence on *Platanus occidentalis* in the southeastern and southern central United States, especially in the valleys of the lower Ohio and lower Mississippi rivers. Among the early records of the occurrence of this fungus (under different names) is that of Jennings (*10*) in 1890, in Texas; Tracy and Earle (*23,* p. 116) in 1895, in Mississippi; Patterson (*14,* p. 31) in 1902, in Illinois. Later, Hoffer (*8*) and Pipal (*15*) recorded its occurrence in Indiana. And in 1925, Martin (*11,* p. 380) reported that the conidial stage of this organism on *P. occidentalis* had been collected in Arkansas, Georgia, Illinois, Indiana, Iowa, Louisiana, Mississippi, Missouri, North Carolina, Oklahoma, Texas, and West Virginia.

The writers have examined specimens of *Mycosphaerella polymorpha* from Arkansas, Illinois, Mississippi, Missouri, North Carolina, and Oklahoma, and have found them all to be specifically identical and distinct from *Stigmina Platani* from the Old World and also from *Stigmella Platani-racemosae* from California.

MATERIALS USED

The materials used in these studies were from many different sources. Herbarium specimens of leaves were generously loaned by Claremont College, Claremont, California, and by Dr. D. S. Welch, of Cornell University. Herbarium specimens and information were provided by the Farlow Library and Herbarium, Harvard University; by the University of California Herbarium, Berkeley; and by the University of California Citrus Experiment Station, Riverside. Freshly pressed leaves of *Platanus occidentalis* affected by *Mycosphaerella polymorpha* were received from Dr. Frederick A. Wolf of Duke University; and leaves of *P. orientalis* affected by *Stigmina Platani* were received from Dr. R. M. Nattrass, Mycologist, Nicosia, Cyprus. Leaves of *P. racemosa* affected by *Stigmella Platani-racemosae* were collected by the writers in California.

The species of *Platanus* used in the inoculation experiments were: (1) *P. orientalis,* trees grown from seed sent by Professor P. Th. Anagnostopoulos, Superior School of Agriculture, Athens, Greece; (2) *P. Wrightii* S. Wats., trees grown from seed and cuttings from Dr. R. B. Streets, University of Arizona; (3) *P. occidentalis,* trees grown from cuttings from Dr. Carroll W. Dodge of the Missouri Botanical Garden, and trees from local nurseries; (4) *P. acerifolia* Wild (hybrid), trees from local nurseries; and (5) *P. racemosa,* trees from local nurseries and trees growing on the Citrus Experiment Station campus.

APPEARANCE OF THE DISEASES

Both macroscopic and microscopic differences may be employed in distinguishing these diseases.

Macroscopic Appearance.—Leaf-spot disease on *Platanus racemosa,* caused by *Stigmella Platani-racemosae* (fig. 1, *A–C*), is manifested by the presence of small, effuse, black-colored areas, 1 to 3 mm in diameter, on the lower surfaces of the leaf blades and on the stipules. These areas generally increase in diameter to about ½ cm, but the entire lower leaf surface may become blackened because of numerous secondary infections. The blackening is produced by the abundance of conidiophores and conidia. If the lesions are widely scattered, the spots may gradually enlarge to 1 cm in diameter. The leaf tissues immediately above the fungus (fig. 1, *A*) are at first yellow, but later become brown and necrotic. The margins of the spots are usually definite, irregular, and surrounded by green tissue.

Lesions on *Platanus orientalis* caused by *Stigmina Platani* (fig. 1, *F*), as observed on herbarium material from Cyprus, are very similar in general appearance to the spots on *P. racemosa* caused by *Stigmella Platani-racemosae.* The two diseases can best be distinguished by comparative microscopic examination of the conidiophores and conidia (see "Microscopic Appearance," below.)

Lesions produced by the conidial stage of *Mycosphaerella polymorpha* are at first pale-green, indefinitely limited areas, if viewed from the upper leaf surface. Thin, weftlike gray stippled areas (fig. 1, *D*) cover the corresponding areas on the lower leaf surface. When the disease has progressed to the extent that a large proportion of the upper leaf surface is pale green, the entire lower leaf surface may be invested with an effuse gray coating (fig. 1, *E*) of conidia and conidiophores. At this stage, which may have developed by midsummer, the trees will appear blighted, and defoliation will have begun.

Microscopic Appearance.—Sections of lesions caused by *Stigmella Platani-racemosae* on *Platanus racemosa* show the fungus to be localized

at first in the stomatal chambers (fig. 2, *I*). Later, hyphae are produced that ramify between the cells; these may extend throughout the tissues to the upper epidermis. Mycelia and haustoria were not found within the cells when sought in paraffin sections or in freezing microtome sec-

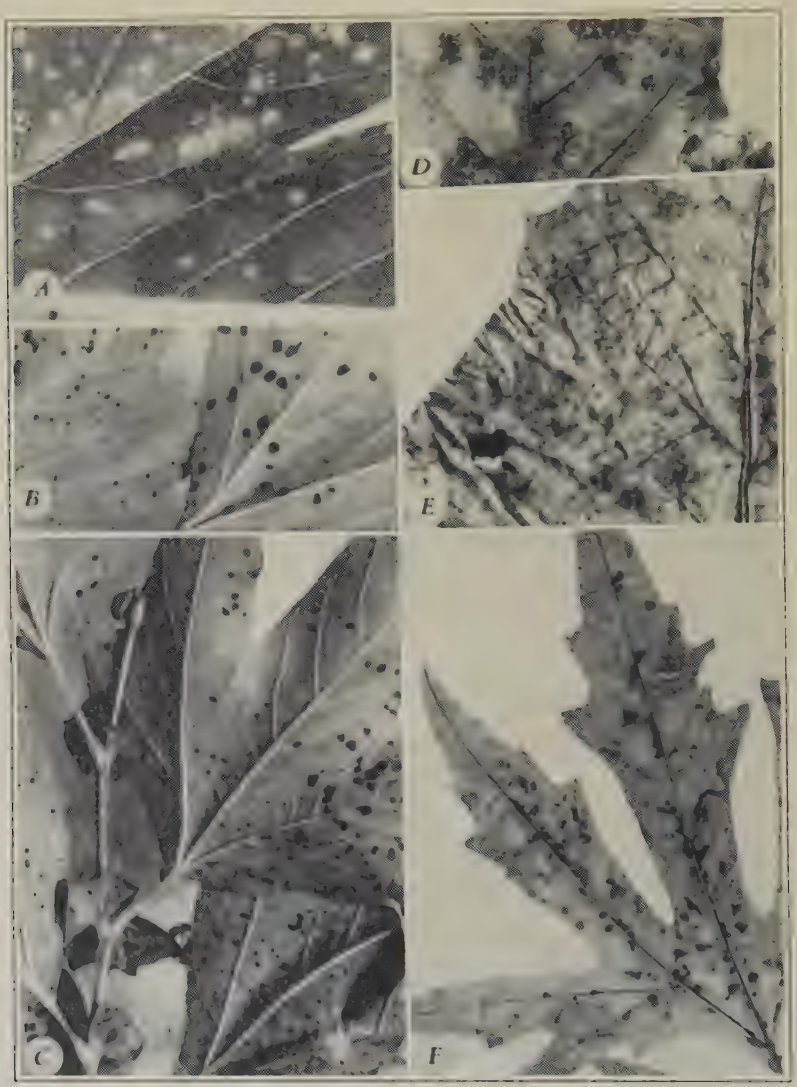

Fig. 1.—Natural infection on leaves of *Platanus* spp.: *A–C, Stigmella Platani-racemosae* on *P. racemosa*—*A*, showing as spots on upper leaf surface; *B*, on lower leaf surface; *C*, on lower leaf surface and on stipules. *D, E, Mycosphaerella polymorpha* on lower leaf surfaces of *P. occidentalis*. *F, Stigmina Platani* on lower leaf surface of *P. orientalis* (from Cyprus).

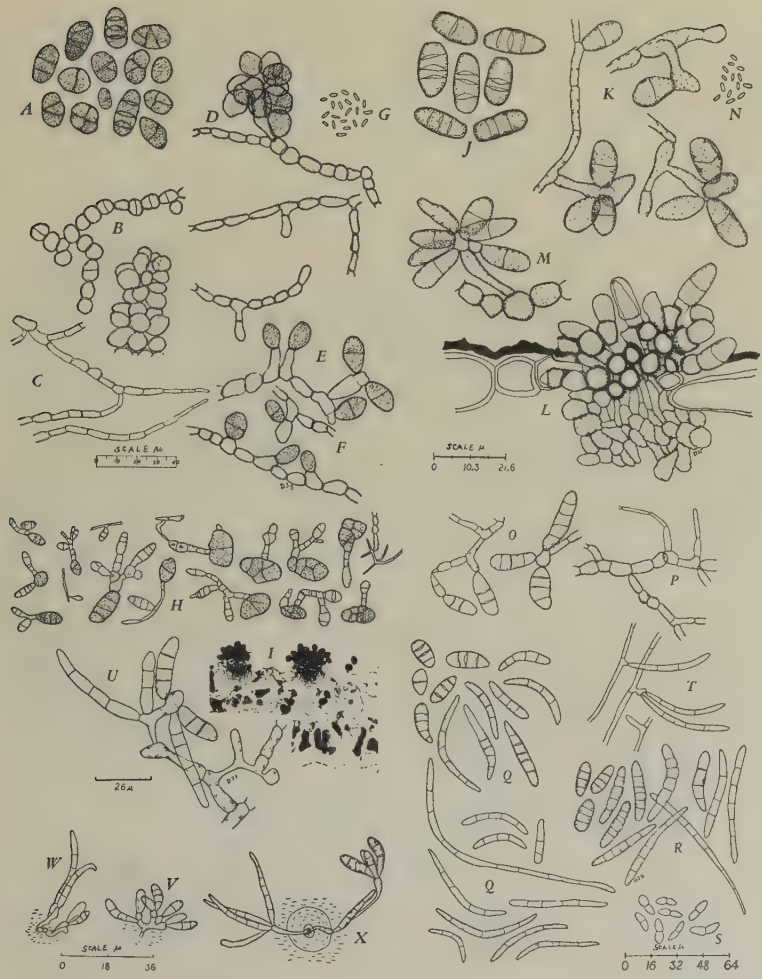

Fig. 2.—Camera-lucida drawings of spores and mycelium of species of *Stigmina* and *Stigmella* on *Platanus* spp.

Stigmella Platani-racemosae from *Platanus racemosa*, *A–I*: *A*, conidia; *B*, mycelium thick, dark and massed; *C*, mycelium hyaline and slender; *D*, attachment of spores to mycelium; *E*, *F*, mycelium, conidia, and conidiophores, showing spore formation; *G*, spermatia from pure culture; *H*, spore germination in drops of water on slides (germinating spores gave rise to additional spores, but attempt at repetition of these results was unsuccessful); *I*, section showing conidia and conidiophores growing out of stomatal chambers (× 305).

Stigmina Platani from *Platanus orientalis*, *J–N*: *J*, spores from type species; *K*, spores and mycelium produced on Czapek's-agar film on slide; *L*, section of living material; *M*, spores, showing attachment; *N*, spermatia from pure culture growing in leaf juice on filter paper.

Mycosphaerella polymorpha from *Platanus occidentalis*, *O–X*: *O*, *Stigmina*-type spores from culture from single-spore isolation of *Cercospora* type, growing in leaf juice on slide, after 21 days; *P*, mycelium from same culture; *Q*, *Cercospora*- and *Stigmina*-type spores from an infection on *P. racemosa* by an isolate from a single *Cercospora* spore, after 56 days; *R*, dark-colored, subhyaline spores of *Stigmina* and *Cercospora* types from culture from single-spore isolation of *Stigmina* type on Czapek's agar; *S*, ascospores from a dead leaf; *T*, *Cercospora*-type spores from Czapek's-agar film on slide, after 21 days; *U*, sporulation, on slide, of growth from a single *Cercospora* spore, showing variation in spores attached to a conidiophore; *V–X*, spores from artificial inoculation on leaf of *P. acerifolia* (the culture used in this inoculation was from a single *Cercospora*-type spore from a single-spore-culture inoculation that had fruited only *Cercospora*-type spores)—*V*, showing variation in spores attached to a conidiophore; *W*, different types of spores attached to single hypha; *X*, mycelial growth from base of a leaf hair and two types of spores.

tions stained with the differential stains, safranin light-green, triple stain, or iron-alum—hemotoxylin, applied according to methods outlined by Rawlins (*16*). Conidia are produced singly on conidiophores from fascicles projecting through the stomata. Affected leaf tissue shows fewer chloroplasts than normal tissue.

A study of freehand sections of leaf spots caused by *Stigmina Platani*

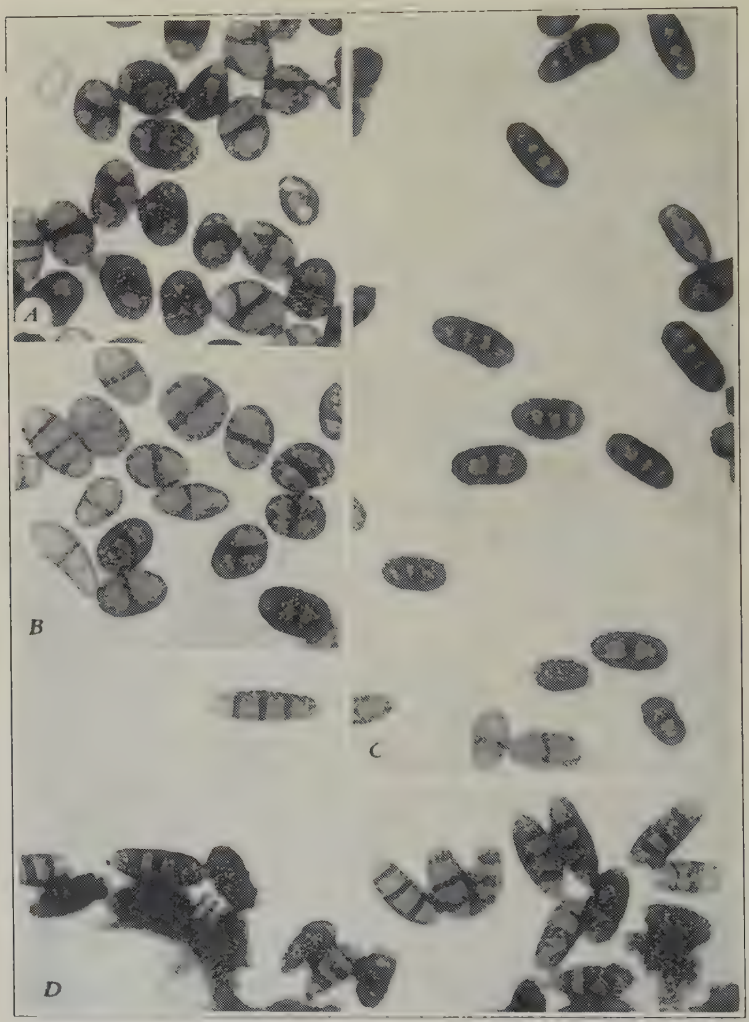

Fig. 3.—Photomicrographs of conidia of the three species of fungi: *A, B. Stigmella Platani-racemosae* from natural infection on *Platanus racemosa*, Riverside, California; *C, Stigmina Platani* from *P. orientalis* from Nicosia, Cyprus; *D, Stigmina* sp. (*Mycosphaerella polymorpha*) from Fungi Columbiana no. 2885 on *P. occidentalis* collected at Rogers, Arkansas. (All × 667.)

on *Platanus orientalis* (fig. 2, *L*) and by *Mycosphaerella polymorpha* on *P. occidentalis* indicated that the relations of the pathogens to the diseased tissues in these species are similar to those found in *P. racemosa*.

The conidia of the three fungi, while somewhat similar in appearance, are sufficiently distinct to suggest three different species. The *Stigmina* type of conidia are oblong, dark-colored, septate bodies (fig. 3, *C*). The *Stigmella* type differed in having numerous irregular septations (fig.

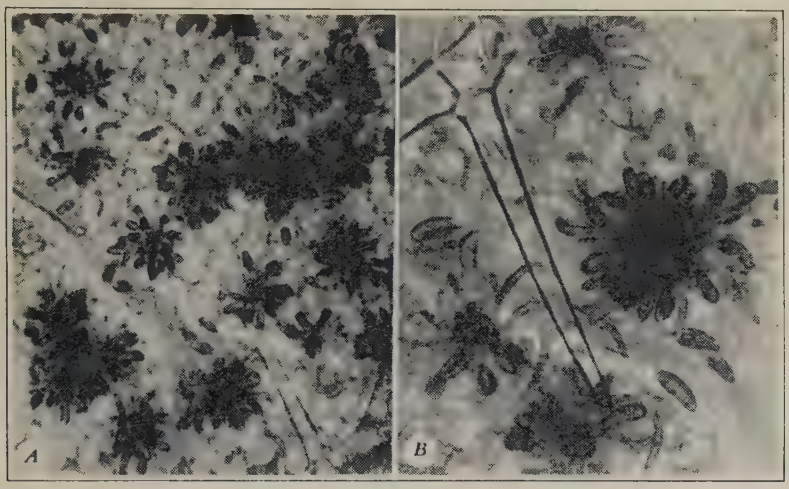

Fig. 4.—Fruiting of fungus on leaf of *Platanus racemosa*, from artificial inoculation with conidial spores of *Mycosphaerella polymorpha* taken from a diseased leaf of *P. occidentalis* from North Carolina: *A*, *Stigmina*- and *Cercospora*-type conidia shown in the same spore clusters (×149); *B*, fruiting of fungus (×305).

3, *A*, *B*). The conidia of *Mycosphaerella polymorpha* were variable in size and shape, as illustrated in figure 4 and in figure 2, *Q*, *R*, *T*, but often the *Stigmina* type (fig. 3, *D*) were the only ones to be found.

MORPHOLOGICAL COMPARISON OF THE THREE FUNGI

Conidial spores of the three fungi, *Stigmella Platani-racemosae*, *Stigmina Platani*, and *Mycosphaerella polymorpha*, taken directly from their respective primary hosts, show wide variation in size and septation (table 1 and fig. 2). While individual spores cannot always be identified with certainty as belonging to one or the other of these three species, conidia of each species, en masse, in spite of variations in septation and size, are sufficiently distinctive to make identification possible.

Conidia of *Stigmella Platani-racemosae* (figs. 2, *A*, and 3, *A*, *B*) are ovate to oblong, 10–22 × 7–13 μ. Septations range from one to three cross

TABLE 1

COMPARATIVE SIZE AND SEPTATION OF SPORES OF SPECIES OF *Stigmella* AND *Stigmina* FOUND ON *Platanus* SPP.

Species and source	Predominant type of spore*	Spores measured	Average			Minimum and maximum			Irregularly septate cells†
			Cells	Length	Width	Cells	Length	Width	
		number	*number*	*microns*	*microns*	*number*	*microns*	*microns*	*number*
Stigmella Platani-racemosae from California	*Stigmina*	35	3.48	16.84	10.64	1–5	10–22	7–13	9
Stigmina Platani from Cyprus	*Stigmina*	50	2.94	18.77	8.75	2–4	14–24	7–11	1
Stigmina sp.‡ from North Carolina	*Stigmina*	51	4.40	22.24	8.91	3–9	14–45	7–11	2
Stigmina sp.‡ from North Carolina	*Cercospora*	52	5.23	47.78	4.54	3–7	17–70	3– 6	0
Stigmina Visianica‡ from Illinois	*Stigmina*	35	5.23	24.21	8.55	2–6	13–34	7–10	1
Stigmina Visianica‡ from Illinois	Intermediate	6	5.83	34.00	7.46	4–7	26–43	6– 9	0

* *Stigmina*-type spores are oblong, dark-colored, and septated (figs. 2, *O*, and 3, *D*); *Cercospora*-type spores are crescent-shaped, septated, and nearly hyaline (fig. 2, *T*); the "intermediate" type are those intermediate in shape between the *Stigmina* and *Cercospora* types.

† Irregularly septate spores are those that have a cross wall and other septa at an angle to the cross wall (fig. 2, *A*)—a muriform type of septation characteristic of *Stigmella*.

‡ Now considered to be a conidial stage of *Mycosphaerella polymorpha*.

septa or from one to four diagonal or irregular septations. The abundant, irregularly septate spores distinguish this fungus from the fungi on *Platanus orientalis* (figs. 2, *J*, and 3, *C*) and on *P. occidentalis* (figs. 2, *O–T*, and 3, *D*).

Conidia of *Stigmina Platani* from *Platanus orientalis* are narrower, and some are longer than those of *Stigmella Platani-racemosae*, as indicated by the dimensions, 14–24 × 7–11 μ, given in table 1. These spores rarely show the irregular septa characteristic of *Stigmella*.

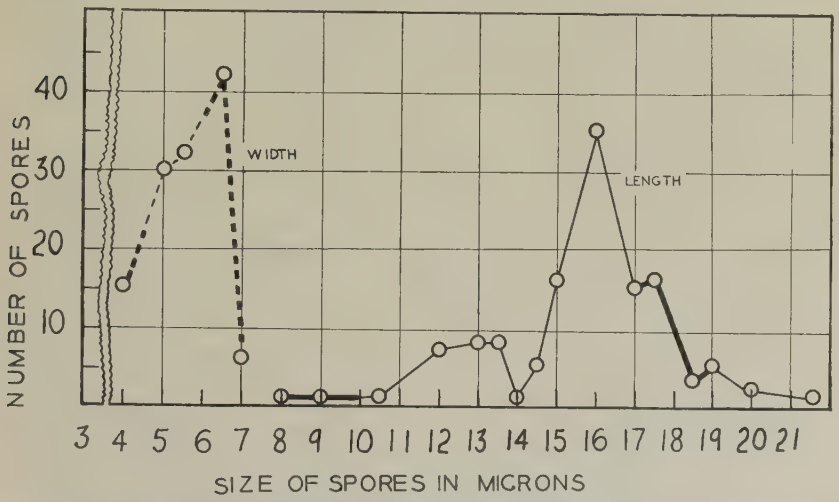

Fig. 5.—Frequency-distribution curves of measurements of 125 ascospores of *Mycosphaerella polymorpha*. Heavy lines indicate measurements corresponding with those of Wolf's (*24*) description of what he called *M. platanifolia* and *M. Stigmina-Platani*.

The *Stigmina*-type spores of *Mycosphaerella polymorpha* (figs. 2, *O*, and 3, *D*) are more elongate and have more acute ends than those of the two other species. They are oval, dark, thick-walled, and have been found to measure 14–45 × 7–11 μ (table 1). *Cercospora*-type spores are still more elongate, are light brown to dark brown in color, thinner-walled, and usually curved or crescent-shaped (fig. 2, *T*); they measure around 17–70 × 3–6 μ, although spores 120 μ in length have been found. Between these two types there are other spores intermediate in shape and size.

One hundred and twenty-five ascospores of *Mycosphaerella*, ejected from leaves into drops of distilled water suspended on the inside cover of a petri dish, were measured under an oil immersion; they ranged in size from 8–19 × 4–7 μ. (Ascospores from the same leaves were ejected onto agar media for culture studies; see p. 219.)

A frequency-distribution curve (fig. 5) shows a number of spores of an intermediate size that apparently bridges the gap between the spore

sizes of *Mycosphaerella polymorpha* and *M. platanifolia* (Cke.) Wolf (*24*). Wolf[7] states, however, that two population curves are shown here (fig. 5), and that measurements of a larger number of spores should confirm this.

PATHOGENICITY

Inoculations of *Platanus* spp. with fungi of *Stigmella* and *Stigmina* spp. were made by the following procedures. (1) Conidia from the leaves or from pure cultures of the fungi were suspended in water. The fungus suspension was applied to the leaves and young shoots by means of either a camel's-hair brush or an atomizer. In nearly all of these tests, a paraffin-paper or cellophane bag or a bell jar was used as a covering to maintain moisture conditions favorable for infection. This protection also prevented fortuitous dissemination and spread of the fungi used in the inoculations. The paper bags (fig. 6, *C*) were not sealed but had their edges twice-folded and kept in place by means of paper clips. In preliminary experiments, it was found that injuries, such as perforations made with a pin, were not necessary for infection, for infections seldom occurred in the loci of injuries but were common between them. (2) Infected leaves were pinned to normal leaves, which were then covered with paraffin-paper or cellophane bags. This method was very satisfactory in moist weather. (3) Spores and bits of mycelium from single-spore cultures of each of the three organisms were placed on leaves enclosed in cellophane bags. Each of the three causal organisms was later recovered in pure culture from the artificially inoculated leaves. Results of the tests are given in table 2.

The fungus *Stigmella Platani-racemosae* caused infection and spread rapidly both on *Platanus Wrightii* (fig. 6, *E* and *F*) and on *P. racemosa* (fig. 6, *G–I*). So far as can be determined, this fungus has not previously been reported on *P. Wrightii*. It failed repeatedly, however, to produce infection when inoculated on *P. orientalis, P. occidentalis,* and *P. acerifolia* (hybrid trees).

Inoculations with *Stigmina Platani* (Cyprus strain) from *Platanus orientalis* resulted in infection on *P. orientalis* (fig. 6, *A*), but were ineffective on *P. racemosa, P. Wrightii, P. occidentalis,* and *P. acerifolia.*

Ascospores and conidia of both *Stigmina* and *Cercospora* types of *Mycosphaerella polymorpha* from *Platanus occidentalis* produced infection on *P. occidentalis* (fig. 6, *D*), *P. racemosa, P. Wrightii,* and *P. acerifolia,* but no infections developed on *P. orientalis.* All inoculations with cultures from single conidia of the *Stigmina* type of *Mycosphaerella polymorpha* produced some spots on which the *Cercospora*-type conidia were

[7] Wolf, F. W. In letter to the senior author dated October 17, 1938.

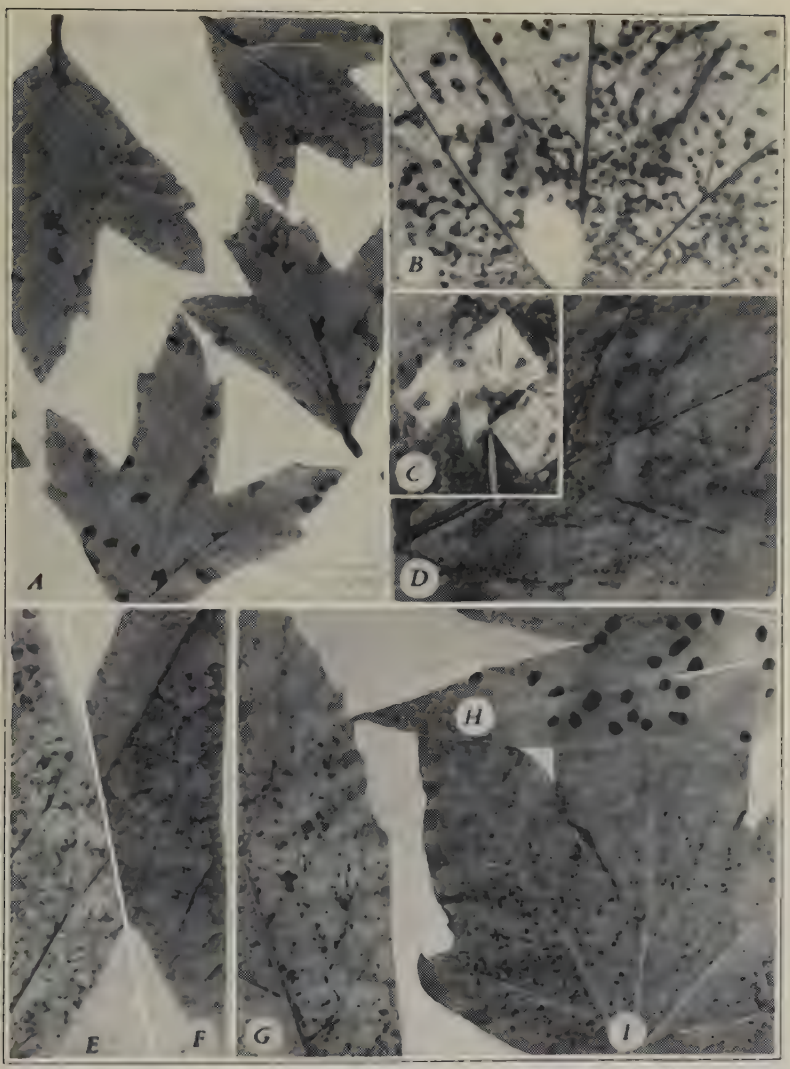

Fig. 6.—Artificial inoculations on species of *Platanus: A, Stigmina Platani* on young leaves of *P. orientalis; B, Mycosphaerella polymorpha* (from natural infection) on *P. racemosa; C*, inoculated leaves protected by paraffin paper bags; *D, Mycosphaerella polymorpha* (culture from a single *Stigmina* type spore isolated from *P. occidentalis* from North Carolina) on authentic leaf of *P. occidentalis*, where *Stigmina* and *Cercospora* type spores fruited in abundance; *E–I, Stigmella Platani racemosae*—*E* and *F*, on upper and lower surfaces, respectively, of young leaf of *P. Wrightii*, after one month; *G*, on succulent leaf of *P. racemosa*, after one month; *H*, on normal leaf after three months; and *I*, after one month.

found. Most inoculations with *Cercospora*-type conidia produced some spots in which the *Stigmina*-type conidia were found. Usually a mixture of the two forms of conidia, together with conidia intermediate in form, was found on lesions resulting from single-spore inoculations. In some cases the fascicles bore *Stigmina*-type conidia only; in others, conidia of the *Cercospora*-type only; and in others, both types were formed on one and the same fascicle. Variations in conidia from single-spore cultures are shown in figure 2, *U–X* (p. 211).

TABLE 2

RESULTS OF INOCULATIONS OF *Platanus* SPP. WITH FUNGI OF *Stigmella* AND *Stigmina* SPP.

Host	Single-spore inoculations					
	Stigmella Platani-racemosae from *Platanus racemosa*		*Stigmina* sp.* from *Platanus occidentalis*		*Stigmina Platani* from *Platanus orientalis*	
	Positive results	Negative results	Positive results	Negative results	Positive results	Negative results
Platanus acerifolia	0	5	8	0	0	8
P. occidentalis	0	9	4	3	0	10
P. orientalis	0	7	0	5	3	1
P. racemosa	10	0	10	5	0	8
P. Wrightii	7	0	5	3	0	4

* Conidial stage of *Mycosphaerella polymorpha.*

The minimum time from inoculation to the beginning of sporulation was about three weeks. In the cooler spring months, the period of incubation was from four to five weeks.

When the surface of leaves atomized with conidia of *Stigmella Platani-racemosae* was stained with safranin, germ tubes entering the stomata could occasionally be found. When surface growth on very young spots was removed, the substomatal areas were observed to be darkened. The absence of darkening elsewhere indicated that the fungus growth was confined to the stomatal areas. Stained paraffin sections of very young spots also showed the fungus localized in the stomatal cavities, with no growth elsewhere (fig. 2, I, p. 211). Infection was often abundant on the lower surface of uninjured leaves of *Platanus racemosa* that had been atomized with a spore suspension. These microscopic observations indicate that the pathogen penetrates by way of the stomata. Similar results were obtained with *Stigmina Platani* and *Mycosphaerella polymorpha;* so that each fungus appears to have the same mode of entrance into the leaves and the same type of subsequent growth within the substomatal tissues.

If susceptibility to infection is an index of relationship, artificial inoculations indicate that *Platanus racemosa* and *P. Wrightii* are closely related and that *P. occidentalis* is more closely related to the American species of *Platanus* than to the European, *P. orientalis*.

CULTURE STUDIES

Methods and Media.—The fungi were isolated in single-spore cultures by streaking suspensions of conidia on the surface of agar plates, where they could be observed in position under the high power of a binocular microscope; then by proper manipulation, single conidia could be picked up with a sharp needle and transferred to media in test tubes. Cultures from ascospores were obtained on agar in petri dishes inverted over moistened dead leaves, from the surface of which the perithecia protruded and discharged the spores onto the surface of the agar.

The media used, in the order of decreasing suitability for sporulation, were as follows: (1) *Platanus racemosa* leaf juice sterilized by filtration, (2) a special medium described by Smith and Smith (*21*) and made by the aseptic addition of an equal amount of the leaf juice to 4 per cent Czapek's agar, (3) Czapek's agar, (4) leaf-extract agar containing 3 per cent sucrose, (5) glucose potato agar, and (6) carrot plugs.

Filtered leaf juice was used both on slides supported on U-shaped glass rods in petri dishes and in Van Tieghem cells, for comparison of the sporulation of isolates from different types of spores. Living leaves were used for certain tests.

Results.—By these procedures, single-spore cultures of *Mycosphaerella polymorpha* were isolated as follows: 147 from *Cercospora*-type conidia, 165 from *Stigmina*-type conidia, and 76 from ascospores. Results of the tests are presented in table 3.

In other tests (not reported in table 3), 8 transfers from ascospore cultures of *Mycosphaerella polymorpha* onto Czapek's agar yielded 5 cultures that produced both *Stigmina*- and *Cercospora*-type spores, 1 culture that produced only *Cercospora*-type spores, and 2 that did not sporulate. Three isolates of *Cercospora*-type spores, when grown on Czapek's agar, yielded 2 cultures that produced only *Stigmina*-type spores and 1 that produced both *Stigmina*- and *Cercospora*-type spores.

Single-spore isolations of *Mycosphaerella polymorpha,* whether from conidia or from ascospores, produced on Czapek's agar two general types of colonies (fig. 7) about equal in number. One type was flat, light mouse gray to mouse gray (*17*); the other type was elevated and smaller in diameter than the first type under the same growing conditions. The color of the latter type was a similar gray, some colonies having white areas, however, and black margins (fig. 7). With age, a light-yellow

color sometimes appeared. When viewed from beneath, both types of colonies appeared dark olive-gray to olivaceous black (*17*). Colonies produced on glucose potato agar were usually much elevated and gray to black in color. On a basis of minor differences, the colonies could be

TABLE 3

SPORULATION OF *Mycosphaerella polymorpha* FROM SINGLE-SPORE ISOLATIONS IN CULTURE MEDIA AND ON LEAVES OF *Platanus racemosa*

Type of spore isolated and medium inoculated	Total inoculations	Infections from different types of sporulation		
		Cercospora-type	*Stigmina*-type	Mixed, *Cercospora* and *Stigmina**
	number	*number*	*number*	*number*
Cercospora:				
Living leaves (March, 1938)	1	1	0	0
Living leaves (April, 1938)	1	1	0	0
Living leaves (May, 1938)	4	0	1	3C
Living leaves (June, 1938)	9	3	1	5C
Living leaves (September, 1938)	12	0	1	5C and 6S
Czapek's agar	16†‡	7	0	8C
Leaf juice on slides	3	2	0	1C
Leaf juice in Van Tieghem cells	6	0	0	6C
Stigmina:				
Living leaves (September, 1938):	11	0	0	6C and 5S
Czapek's agar	16†	10	0	6C
Leaf juice in Van Tieghem cells	6	0	0	6C
Ascospore:				
Living leaves (May, 1938)	15	10	0	5C
Czapek's agar	74‡	72	0	0
Leaf juice on slides	8‡	1	0	5C
Leaf juice in Van Tieghem cells	4‡	2	0	0

* The letters "C" and "S" indicate the type of spore (*Cercospora* or *Stigmina*, respectively) predominating.

† Isolates selected at random.

‡ Some of these inoculations failed to produce spores.

grouped into no less than fourteen different types. While occasional differences were noted in the size of spores produced by the different isolates, these variations could not be correlated with the kind of spore from which the culture originated.

Spermatia (fig. 2, *G* and *N*) of each of the three organisms developed both in cultures and on infected leaves. In cultures of *Mycosphaerella polymorpha,* dense mycelial masses developed containing these small bacilluslike bodies. But when these were streaked on nutrient media, no evidence of spermatial germination was obtained.

Of the 50 single-spore cultures of *Stigmella Platani-racemosae* made in these tests, 1 isolate produced a flat, spreading colony, when grown on

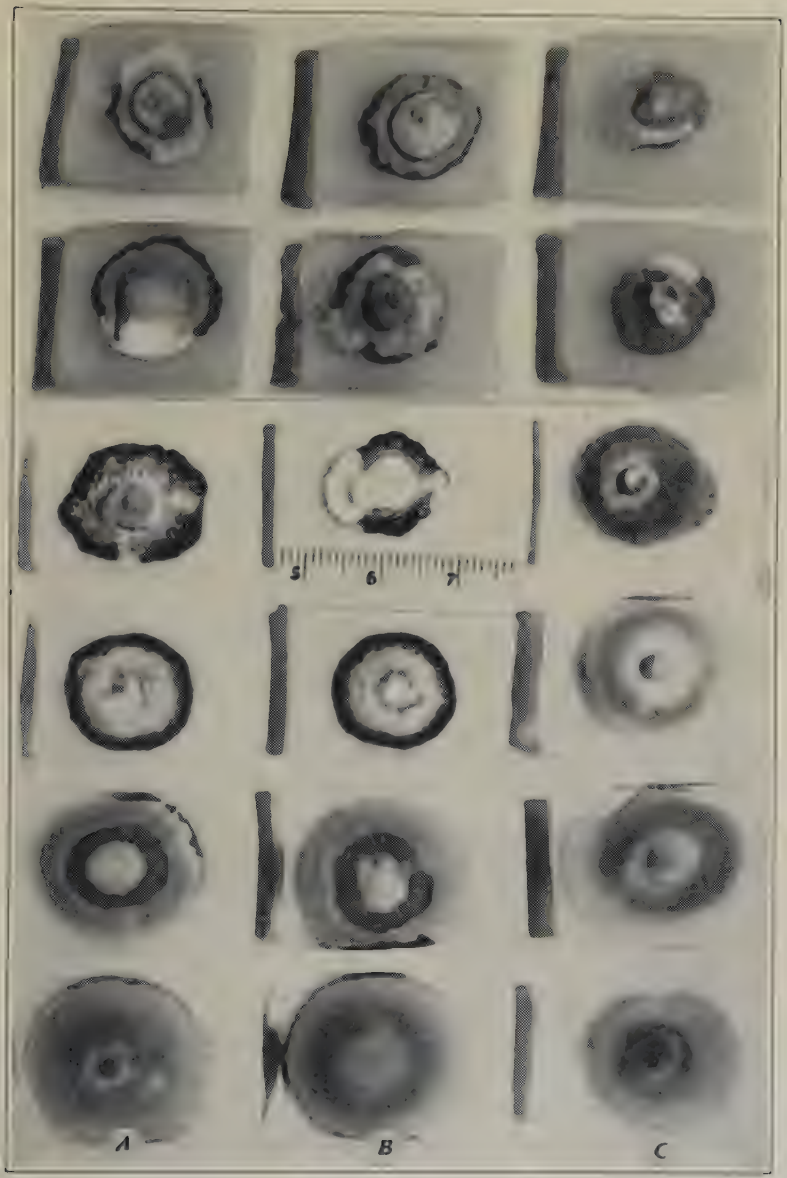

Fig. 7.—Colonies grown on Czapek's agar from single-spore culture isolations (different spore types) of *Mycosphaerella polymorpha*. The vertical rows show colony variation: *A*, colonies from *Cercospora*-type spores; *B*, from *Stigmina* type spores; *C*, from ascospores. The horizontal rows show colonies of similar types of growth.

glucose potato agar at room temperature; the other 49 produced colonies that were elevated.

The colonies of *Stigmina Platani* closely resembled those of *Stigmella Platani-racemosae* but were less variable than those of *Mycosphaerella polymorpha.*

TEMPERATURE RELATIONS

The effects of temperature on these three species of fungi were determined by the use of constant-temperature chambers having a range of 10°–35.5° C. A few tests were also made in a refrigerator at 5°. The

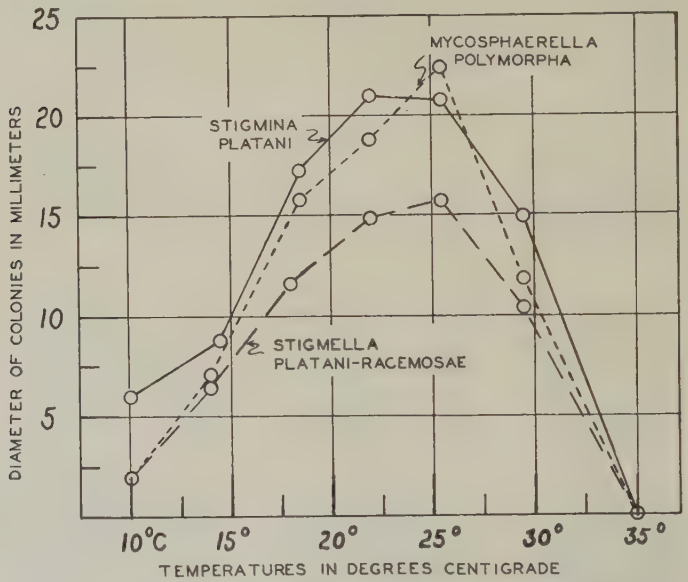

Fig. 8.—Growth response of colonies grown on glucose potato agar (pH 4.7) at different temperatures: *Stigmina Platani,* six weeks; *Stigmina* sp. (*Mycosphaerella polymorpha*), one month; *Stigmella Platani-racemosae,* one month.

diameter of colonies grown on glucose potato agar was used as an indicator of mycelial growth response. The optimum for all three organisms (fig. 8) was between 22° and 26°. They showed slight growth below 10°, but failed to grow at 35.5°. The growth curves of *Stigmella Platani-racemosae* and *Stigmina Platani* are flatter near the optimum than that of *Mycosphaerella polymorpha. Stigmella Platani-racemosae* gave the best sporulation between 14° and 19°; the other two fungi did not sporulate within this range during the time of the experiment.

The temperature at which the maximum germination of conidia of *Stigmella Platani-racemosae* and of *Mycosphaerella polymorpha* (fig.

9) took place (conidia of *Stigmina Platani* were not tested) agrees with that of the optimum temperature for growth of the mycelia of these two fungi. At 25° C, there was 75 per cent germination within 28½ hours, and 90 per cent within 47 hours. The count was based upon 50 spores selected at random. The germ tubes varied in length with the temperature; at 25° they reached an average length of 15.5 μ in 47 hours.

The thermal death points of the mycelia and spores of the three fungi were tested with cultures and conidia taken directly from the leaves of their respective hosts. Mycelium obtained from nonsporulating colonies

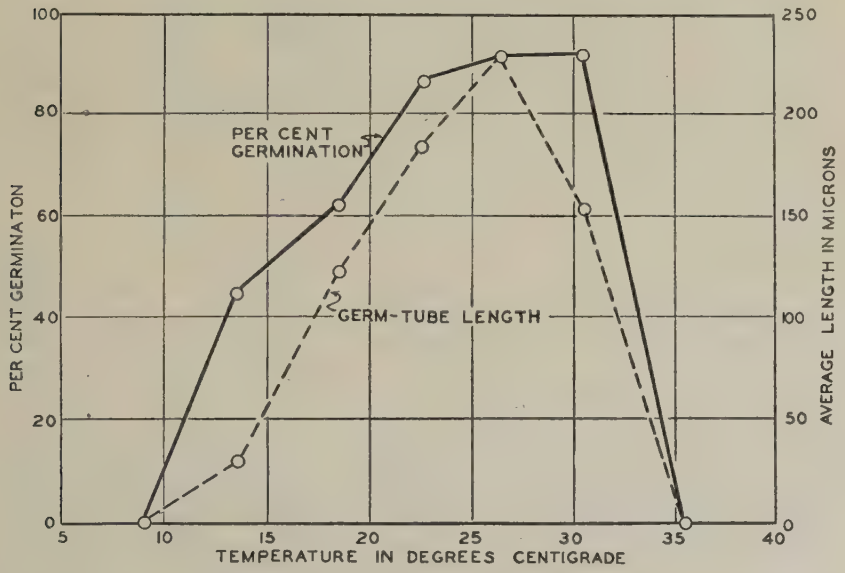

Fig. 9.—Spore germination and germ-tube growth of *Stigmina* sp. (*Mycosphaerella polymorpha*) on glucose-potato-agar film on slides for 48 hours at different temperatures.

was broken into bits by means of a sterile rod. Concentrations of the suspensions of mycelia or conidia were adjusted by the addition of sterile water. These suspensions were put into sterile capillary tubes, 4 to 5 inches long, after which the tubes were sealed by means of a microburner. The temperature of the bath, which consisted of a large pan of water, was modified by means of a microburner. After exposure for a 10-minute period in the bath, the capillary tubes were surface-sterilized in mercury bichloride and washed in sterile water; the contents were then ejected upon glucose-potato-agar plates. Observations of the resultant growth were made at intervals of from one day to two weeks. The results of these tests are shown in table 4.

The thermal death point of mycelia and spores of both *Stigmella Platani-racemosae* from *Platanus racemosa* and *Stigmina Platani* from *P. orientalis* was found to lie between 45° and 46.5° C. The mycelium and spores of the *Stigmina* stage of *Mycosphaerella polymorpha* had a thermal death point between 47° and 48°, approximately 2° higher than that of the other two fungi.

Mycosphaerella was cultured in sterile leaf juice in Van Tieghem cells to test the influence of temperature on sporulation. Five cultures were

TABLE 4

DETERMINATION OF THERMAL DEATH POINT OF MYCELIUM AND SPORES OF THE THREE FUNGI

Fungus growth treated	Effect of 10-minute treatments at different temperatures*						
	44° C	45° C	46° C	46.5° C	47° C	48° C	49° C
Stigmella Platani-racemosae from *Platanus racemosa:*							
Spores	+	+	+	−	−	−	
Mycelium	+	+		−		−	
Stigmina stage of *Mycosphaerella polymorpha* from *P. occidentalis:*							
Spores	+	+	+		+	−	−
Mycelium	+	+	+		+	−	−
Stigmina Platani from *P. orientalis:*							
Spores	+	+	−		−		−
Mycelium	+	+		−		−	

* In the columns + = continued fungus growth indicated; − = no growth and probable death of the organism; blank spaces indicate that results at these temperatures were not recorded. For description of treatments see text (p. 223).

maintained at 15° C, 5 at 19°, 16 at room temperature (21°–33°), 5 at 26.5°, and 5 at 30.5°. Of the 16 cultures grown at room temperature, 6 originating from *Stigmina* spores produced a mixture of *Stigmina*- and *Cercospora*-type conidia; 6 originating from *Cercospora*-type spores also produced conidia of both kinds; 2 of the remaining 4 cultures, which originated from ascospores, produced *Cercospora*-type conidia only, and 2 remained sterile. Sporulation did not occur in any of the other cultures with the exception of one of those maintained at a temperature of 26.5°, which produced *Cercospora*-type conidia two weeks after inoculation.

Cercospora-type conidia were often found in cultures from the conidia (both *Cercospora*- and *Stigmina*-type) and ascospores of *Mycosphaerella polymorpha*, but were never found in cultures of *Stigmella Platani-racemosae* or of *Stigmina Platani.*

Results of inoculations with cultures from single spores on leaves of *Platanus racemosa* would seem to indicate that temperature may be a factor in determining the type of spore that will develop on the infected

leaf tissue (table 5). The production of the *Cercospora*-type was favored by the higher temperatures, and the *Stigmina*-type developed best at the lower temperatures.

HYDROGEN-ION RELATIONS

The hydrogen-ion relations of *Stigmella Platani-racemosae* and of *Stigmina Platani* were tested in carrot dextrose broth (fig. 10), on Czapek's agar, and on carrot dextrose agar. *Mycosphaerella polymorpha*

TABLE 5

SPORULATION FROM SINGLE-SPORE INOCULATIONS ON LEAVES OF *Platanus racemosa* WITH SPORES OF *Mycosphaerella polymorpha* UNDER DIFFERENT TEMPERATURE CONDITIONS; NOVEMBER—DECEMBER, 1938

Type of spore isolated and culture no.	Lathhouse, temperature variable*		Cool greenhouse, temperature 21°–27° C		Warm greenhouse, temperature 27°–32° C	
	Inoculations	Sporulation†	Inoculations	Sporulation†	Inoculations	Sporulation†
	number		*number*		*number*	
Ascospore:						
No. 5	1	S only	1	None	1	None
No. 11	1	S and C equal	1	None	1	None
No. 17	1	S only	1	None	1	None
No. 22	2	S only	2	None	2	C only
No. 23	2	None	2	None	2	C only
No. 34	2	Mixed, S predominant	1	S and C equal	1	C only
Stigmina-type:						
No. 227	1	S only	1	None	1	None
No. 239	1	None	1	None	1	Mixed, C predominant
Cercospora-type						
No. 115‡	1	None	1	Mixed, C predominant	1	None
No. 118	1	None	1	None	1	None
No. 130	1	None	1	None	1	Mixed, C predominant
No. 136	1	None	1	Mixed, C predominant	1	None

* No temperature records were taken for the lathhouse, but outside temperatures as recorded at the Citrus Experiment Station for November were: minimum, 2° C; maximum, 29°; mean minimum, 4.3°; mean maximum, 24.6°. For December the minimum was 2°; maximum, 32°; mean minimum, 6.5°; mean maximum, 21.9°.

† The letters "S" and "C" indicate the type of sporulation, *Stigmina* or *Cercospora*, respectively.

‡ Organism isolated from inoculation by a previous single-spore culture.

was not tested. The fungi were grown in the liquid medium for three and one-half months and on the solid media for six weeks, at their optimum temperature of 25° C. In the cultures on Czapek's agar and on carrot dextrose agar, the minimum pH value was 2.0, the optimum 5.0, and the maximum between 7.0 and 8.0. Growth was slight at the alkaline end of the range. Growth of the fungi caused increased alkalinity in liquid cul-

tures, whereas the control liquids were more acid at the end than at the beginning of the experiment (fig. 10). There was little change in the pH value of the media where the initial concentration was pH 7.0.

INITIAL PH VALUE	ORGANISM	FUNGUS GROWTH IN GRAMS
2.08	STIGMELLA	0.333
	STIGMINA	0.257
3.64	STIGMELLA	0.302
	STIGMINA	0.392
4.22	STIGMELLA	0.253
	STIGMINA	0.440
5.05	STIGMELLA	0.450
	STIGMINA	0.416
6.02	STIGMELLA	0.390
	STIGMINA	0.328
6.48	STIGMELLA	0.362
	STIGMINA	0.364
7.15	STIGMELLA	0.245
	STIGMINA	0.230
8.30	STIGMELLA	0.302
	STIGMINA	0.220

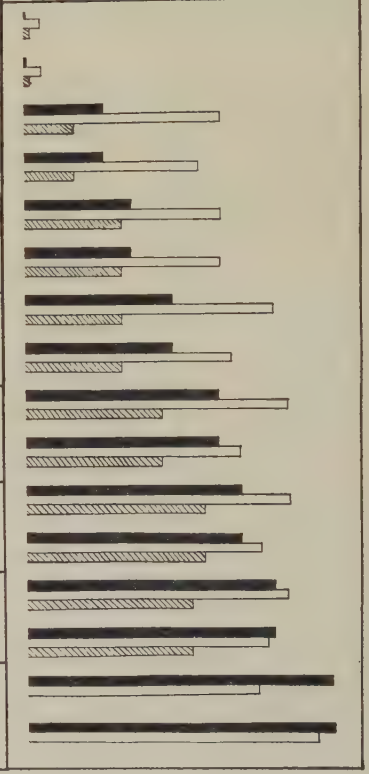

Fig. 10.—Hydrogen-ion relations of *Stigmella Platani-racemosae* and of *Stigmina Platani* grown for three and one-half months on the surface of 100 cc of carrot dextrose broth in 200-cc flasks at 25° C. Control liquids contained no fungus inoculation.

Germination of spores and germ-tube length of *Stigmella Platani-racemosae* at different pH values, when tested in carrot-dextrose-agar drops on slides for 28 hours, showed maximum germination of approximately 85 per cent at pH 5.0. The germ-tube length, however, was great-

est at pH 4.5. At pH 5.0, the average germ-tube length was decreased by about one half of that at pH 4.5. The juice of leaf blades of *Platanus racemosa* had a pH value of 4.8 in April, as determined by the quinhydrone glass electrode; that of *P. acerifolia* gave a reading of pH 5.1 in September; there is, therefore, a close correlation between the pH of the leaf juice and that which is optimum for the pathogen.

CONTROL

The writers have conducted no experiments for control of the leaf spot on *Platanus racemosa.* The other two leaf spots are not found in California. Should control be found necessary, treatment with bordeaux mixture, as suggested by Felt and Rankin (*6*) in the control of *Gnomonia veneta* (Sacc. and Speg.) Kleb., which causes leaf and twig blight of plane tree, or sycamore, should be satisfactory. These writers recommend that the trees be sprayed with bordeaux mixture after the buds burst and before the leaves are half grown, and that a second application be given one week later. A third and fourth spraying at intervals of two weeks are suggested if the season is rainy. It is anticipated that burning the fallen leaves would lessen the severity of the initial infections and might well be a very important control measure.

DISCUSSION AND SUMMARY

On the basis of morphological and physiological differences and of host specificity, it is shown that three distinct species of fungi are involved in leaf spots on *Platanus* (plane tree). Each produces a different symptom complex and has a distinct geographical host range.

Stigmina Platani (Fckl.) Sacc. on *Platanus orientalis* L., from Europe, failed to infect other species of *Platanus.*

Stigmella Platani-racemosae Dearn. and Barth. is pathogenic to *Platanus racemosa* Nutt. in California. In pathogenicity tests it proved to be capable of infecting *P. Wrightii* S. Wats., not previously known to be susceptible. Other species of *Platanus* proved to be immune.

The fungus called, in this paper, *Mycosphaerella polymorpha* and found on *Platanus occidentalis* L., occurs in the southeastern and southern central United States. It was found to be pathogenic also on *P. racemosa, P. Wrightii,* and *P. acerifolia,* but not on *P. orientalis.* This organism produces polymorphic conidia that range in shape from those typical of *Stigmina* on the one hand, to those typical of *Cercospora* on the other. Conidia of each type and also of intermediate types may be borne on the same conidiophoral fascicle. This conidial stage has hitherto not been named, for the reason that it has been erroneously identified as *Stigmina Platani.*

The proper denomination of these three fungi could only be accomplished if their perfect stages could be developed under artificial conditions or were found to exist in the natural state on decaying leaves. The writers attempted unsuccessfully to induce the development of the perithecial stage of *Stigmella Platani-racemosae* under California conditions. Furthermore, at the request of the writers, leaves infected with *Stigmina Platani* were maintained in Cyprus under natural conditions, to permit the development of the perfect stage, but to no avail. Each of these organisms probably possesses a perithecial stage. Evidence for this is found in the fact that each possesses a spermatial stage, as previously noted (see "Culture Studies," p. 220). In the light of our knowledge of other ascomycetes, the production of spermatia may properly be interpreted as indicative of the presence of perithecia in the developmental cycle. The fact that these organisms can survive from year to year as conidial stages shows that the perfect stage is not essential to survival; but this is not proof of the nonexistence of a perfect stage.

The perithecial stages of *Mycosphaerella polymorpha* and *M. platanifolia* indicate that these species may be identical. The measurements for freshly discharged, hence mature, ascospores of *M. Stigmina-Platani* (*M. polymorpha*), given by Wolf (*24*, p. 58), are 17–19 × 6–7 μ; and for those of *M. platanifolia*, 8–10 × 4–4.5 μ. In the present study, the range of measurements of ascospores discharged from perithecia borne in leaves of *Platanus occidentalis* was 8–19 × 4–7 μ, as previously stated (p. 215), and included spores 12.0, 13.5, 14.5, and 16 μ long and 4.8, 5.4, and 5.6 μ wide. These measurements indicate that there are spores intermediate in size between those published by Wolf for the two species of *Mycosphaerella*.

Wolf (*24*, p. 59) mentions two types of colonies: one type isolated from either the conidia or the ascospores of *Mycosphaerella Stigmina-Platani* (*M. polymorpha*) and the other type from either the conidia of *Cercospora platanicola* or the ascospores of *M. platanifolia*. But he states further[8] that the two types of colonies found in the present studies (see "Culture Studies," p. 219) and shown in figure 7 (p. 221) appear to have the same characteristics as those found in his studies.

Differences in appearance of colonies within one and the same species, however, are now known to be characteristic of an increasingly large number of fungi. The evidence in hand at present, therefore, as to the possible identity of *Mycosphaerella polymorpha* and *M. platanifolia* must be regarded as insufficient, and the solution of the problem must be left for future study.

[8] Wolf, F. A. In letter to the junior author dated August 10, 1940.

Unfortunately, Wolf (*24*) used the specific name *Stigmina-Platani* for the *Mycosphaerella* on *Platanus occidentalis,* whereas the present studies establish the fact that the conidial fungus *Stigmina Platani* is specifically distinct and occurs only on *P. orientalis.* This error, if preserved, would add to the nomenclatorial confusion, especially if the perithecial stage of *Stigmina Platani,* when discovered, should happen to be found to belong to *Mycosphaerella,* as this genus is now delimited. It has been deemed advisable, therefore, to reject the name *Mycosphaerella Stigmina-Platani* Wolf, and the new name *Mycosphaerella polymorpha* is proposed in its place.

Stigmina Visianica Sacc. appears to be identical with the conidial stage of *Mycosphaerella polymorpha.*

Each species has been isolated and grown in single-spore culture. Sporulation in culture was best induced by growth on *Platanus* leaf juice sterilized by filtration and on Czapek's agar.

Temperatures within the range of 22° to 26° C were found to be optimal for growth of each of the three species.

Germination of spores and mycelial growth occurred best in media having an acidity of approximately pH 5.0.

Removal of fallen leaves and spraying of the trees may be anticipated to be effective control measures.

ACKNOWLEDGMENTS

The writers wish to express appreciation to Professor William T. Horne, under whose supervision the work was conducted, and to others, whose generous coöperation made these studies possible (see "Materials Used," p. 208). Grateful acknowledgment is made to Dr. H. S. Fawcett for assistance in preparation of the manuscript. Valuable information, criticisms, and suggestions were also given by Dr. F. A. Wolf, Dr. L. J. Klotz, Dr. D. E. Bliss, and Dr. C. L. Shear. Dr. Wolf was especially helpful in the problem of nomenclature. The photomicrographs were taken by Dr. L. J. Klotz.

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